

**Thyroid and Adrenocortical Function
During Critical Illness**

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To

Fiona and Ben

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Declaration

I declare that his thesis is of my own composition,
and the research contained herein is my own original
work. No portion of this work has been submitted in
support of an application for any other degree.

Peter Malcolm Rothwell

30th October 1994

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Abstract

Thyroid and adrenocortical function are altered during critical illness. Some of the mechanisms responsible for the changes in thyroid function have been elucidated, and the majority of patients are considered to be euthyroid. Hence, the Sick Euthyroid Syndrome. However, despite normal or raised free thyroxine levels in many patients, some authors have recommended thyroxine replacement. Two small clinical trials of replacement therapy were inconclusive. Similarly, although total cortisol concentrations usually increase during critical illness and there is no evidence of reduced availability of free hormone, there are anecdotal reports of relative adrenocortical insufficiency in some critically ill patients, and a number of ICU clinicians regularly give low dose steroid replacement.

The extent of changes in concentration of thyroid hormones or cortisol have not been determined in sufficiently large numbers of critically ill patients not receiving confounding medications. It is unclear how the changes relate to type and severity of illness. In particular, neither the expected range of baseline hormone concentrations nor the expected responses to dynamic tests of adrenocortical or thyroid function have been defined at different levels of illness severity. Moreover, the nature of the relationship between the changes in thyroid and adrenocortical function and mortality is unclear.

Aims

The main aims of the thesis were as follows: (1) To determine the ranges of total thyroxine, triiodothyronine, TSH and cortisol concentrations at different levels of illness sever-

ity, and to relate these to mortality; (2) To determine the range of responses to standard dynamic tests of adrenocortical and thyroid function, and relate them to severity of illness and mortality; (3) To determine the relationship between the extent of the changes in total thyroid hormone concentrations during illness and metabolic rate; (4) To accurately determine the prognostic value of measurement of thyroxine, triiodothyronine, TSH and cortisol on admission to an ICU.

The question of whether or not thyroxine or cortisol are deficient during critical illness was not addressed. The main aim was to determine whether or not patients with low total hormone concentrations or impaired responses to dynamic tests had an increased mortality when severity of illness was taken into account.

Summary of Main Findings

Adrenocortical Function: The majority of critically ill patients had plasma cortisol concentrations above the upper limit of normal in health. In keeping with Seyle's General Adaption Theory, plasma cortisol concentration correlated with severity of illness. The normal ranges of plasma cortisol concentration, defined as population mean \pm 2 standard deviations, differed according to the severity of illness. A plasma cortisol concentration above 200 nmol/L would be within the 95% range for a moderately ill patient (APACHE II score <16) whereas a concentration of under 400 nmol/L would be below the expected range in a severely ill patient (APACHE II score >24).

There was evidence of impairment of adrenocortical function in patients suffering from septic shock. Basal cortisol concentrations and cortisol responses to standard and low

dose ACTH stimulation tests were lower than in critically ill controls and the cortisol response to depot ACTH was not maintained.

The overall relationship between mortality and plasma cortisol concentration was linear. However, after taking severity of illness into account, a J-shaped relationship was found. In other words, mortality increased as cortisol concentration increased above normal, but low normal concentrations were also associated with a high mortality. The cortisol response to a standard ACTH stimulation test varied between -100 and 1000 nmol/L. In general, the cortisol response was maintained despite very high basal cortisol concentrations. There was an inverse correlation between the cortisol response and severity of illness. Mortality among patients with cortisol responses of less than 250 nmol/L was 76% [95% CI, 55-91] compared with 31% (95% CI, 19-45) among the remainder. A reduced cortisol response to ACTH might be due to adrenocortical impairment in the context of widespread organ failure. The high mortality reflecting the associated organ failure rather than any insufficiency of cortisol. The higher than expected mortality among patients with low admission cortisol concentrations requires further study.

Thyroid Function: In patients without known preexisting thyroid disease, not receiving treatment with dopamine or steroids, the range of TSH concentrations found using a sensitive assay was broader than that found using similar assays in health. TSH concentrations were subnormal in 15% of patients, a third of whom had concentrations of less than 0.1 mU/L. A further 13% of patients had raised TSH concentrations, despite relatively normal total T4 levels. In the majority of these, TSH concentrations returned to normal on

recovery. TSH concentration correlated inversely with severity of illness and plasma cortisol concentration.

Compared with controls, the TSH response to TRH was reduced or absent in all patients. The response correlated inversely with severity of illness and plasma cortisol concentration. There was a positive correlation between TSH and total thyroxine. Patients with low concentrations of both hormones had small or absent TSH responses to TRH, suggesting that function of pituitary thyrotrophs was suppressed. The thyroid hormone response to TSH was normal or increased compared with healthy controls, suggesting that, despite widespread organ failure, function of the thyroid gland was not impaired. These findings raise the possibility that reduced TSH secretion might contribute to the reduced total thyroid hormone concentrations found during critical illness.

Mortality increased as total thyroxine concentration fell. However, the mortality among patients with low concentrations of total thyroxine was no higher than expected on the basis of their severity of illness, suggesting that the association was not causal. Indeed, metabolic rate was highest in patients with the lowest total thyroxine concentrations. These findings do not support the contention that critically ill patients might benefit from thyroid hormone replacement.

A prognostic index based on admission measurements of cortisol, thyroxine and thyrotropin concentrations was developed using multiple logistic regression analysis. The model obtained predicted outcome of illness with significantly greater accuracy than APACHE II scores.

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Preface

I carried out the research described in this thesis between February, 1989 and August, 1990, whilst I was employed as a Senior House Officer on the Cleveland Hospitals Medical Rotation, and during frequent visits to the ICU over the subsequent two years. My Supervisor during this time was Professor C Edwards, University of Edinburgh.

I acknowledge the help of Dr Zarir Udwadia, a colleague on the intensive care unit who took a number of blood samples on my behalf. I planned and performed the dynamic tests of adrenocortical and thyroid function, measured the APACHE II scores, made the measurements of metabolic rate and performed the studies leading to the protocol for the study of adrenocortical function on an ICU. I collected, analysed and interpreted the data. All the assays detailed in this thesis were performed by the technicians working in the Department of Clinical Chemistry, Middlesbrough General Hospital.

Publications and Presentations Arising From this Thesis

Rothwell PM, Udwadia ZF, Lawler PG. Cortisol response to corticotropin in septic shock. *Lancet* 1991; 337: 582- 583.

Rothwell PM, Udwadia ZF, Lawler PG. Relative adrenal insufficiency in critical illness. *Critical Care Medicine* 1991; 19: 589-590.

Rothwell PM, Udwadia ZF, Lawler PG. Thyrotropin levels predict outcome in critical illness. *Anaesthesia* 1993; 48: 373-376.

Rothwell PM, Lawler PG. Prediction of outcome in intensive care patients using endocrine parameters. *Critical Care Medicine* 1995; 23: 78-83.

Rothwell PM. Physiological scoring systems and audit. *Lancet* 1993; 342: 306 (Letter).

Rothwell PM. Prognostic value of admission thyrotropin levels in intensive care patients. *Intensive Care Society International Meeting, London, 1990.*

Rothwell PM. The cortisol response to ACTH in septic shock. *Intensive Care Society International Meeting, London, 1990.*

Rothwell PM. Predicting outcome in critical illness: an endocrine prognostic index. *Fifth International Symposium on Endocrinology in Anaesthesia and Critical Care Medicine, Berlin, 1991.*

Rothwell PM. Thyroid and adrenocortical function in severe illness. *Western General Hospital Grand Round, Nov 1991.*

List of Abbreviations

ACTH	Adrenocorticotropin
APACHE	Acute Physiology and Chronic Health Evaluation
CBG	Corticosteroid binding globulin
CI	Confidence interval
CRH	Corticotropin releasing hormone
CV	Coefficient of variation
EI	Endocrine Index
EMR	Excess metabolic rate
FTI	Free thyroxine index
FSH	Follicle stimulating hormone
hCG	Human chorionic gonadotrophin
IL	Interleukin
H-P-A	Hypothalamic-pituitary-adrenal
H-P-T	Hypothalamic-pituitary-thyroid
ICU	Intensive care unit
LH	Luteinising hormone
MD	Mean difference
MAP	Mean arterial pressure
NEQAS	National External Quality Assessment Scheme
PBMR	Predicted basal metabolic rate
RIA	Radioimmunoassay
ROC	Receiver operating curve
SD	Standard deviation
rT3	Reverse triiodothyronine
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroid binding globulin
TBPA	Thyroid binding prealbumin
TNF	Tumour necrosis factor
TPN	Total parenteral nutrition
TRH	Thyrotropin releasing hormone
TSH	Thyrotropin

Statistical Analysis

A consistent approach to statistical analysis has been attempted. The technique used for a particular analysis will not be stated, unless it deviates from the protocol set out below. For comparison of parametric data, the 95% confidence intervals of the difference between the means are given. Student's t test is used for proportionate data, and χ^2 tests are used for categorical data, unless the numbers are small, in which case Fisher's exact test is used. The Wilcoxon Rank Sum test is used for comparisons of non-parametric data. Correlation is assessed using Pearson's Product Moment method, and multiple regression analysis is performed using Cox's Proportional Hazards method.

Chapter 1

Introduction

- (1) Defining Critical Illness
 - (2) Adrenocortical Function During Critical Illness
 - (3) Thyroid Function During Critical Illness
 - (4) Interpretation of Research in Critical illness
 - (5) Ethical Difficulties of Research During Critical illness
-

(1) Defining Critical Illness

Throughout the intensive care literature there are a large number of undefined terms relating to type or severity of illness. Terms such as sepsis, septicaemia, sepsis syndrome, septic shock, organ failure and multi-organ system failure are all frequently used without qualification. Even in recent multicentre trials (Bone et al, 1987; VASSCSG, 1987; Ziegler et al, 1991) different definitions of sepsis, shock, and organ failure were used. This causes confusion, and limits the generalisability of research findings. There are historical reasons why firm definitions are lacking. Only in the past thirty years have septic shock and multiple organ failure been regularly encountered. Prior to this period it was not possible to keep severely ill patients alive long enough for them to develop such syndromes. Early work focused on patients with Gram-negative bacteraemia. However, we

now know that the majority of patients with sepsis are not bacteraemic. The terms sepsis and infection have been used synonymously, the role of invading organisms being considered crucial, but it is now clear that sepsis is related more to the immunological response of the individual than to the invading organism itself. Moreover, a clinical syndrome identical to sepsis occurs as a complication of a number of conditions in which there is no infection.

Sepsis is a very old medical term which derives from the Greek "sepsin" meaning "to make putrid". It refers to a constellation of signs including fever, tachycardia and tachypnoea, usually accompanied by a characteristic haemodynamic disturbance involving an elevated cardiac output and a reduced systemic vascular resistance. This syndrome is associated with release of a considerable array of mediators, including tumour necrosis factor [TNF], various interleukins, platelet activating factor, nitric oxide and many others. The response to administration of TNF to volunteers is identical to the response following administration of endotoxin, namely fever, malaise and myalgia, with tachycardia, increased cardiac output and reduced systemic vascular resistance (Michie et al, 1988; Fong and Lowry, 1991). The release of these same mediators has been demonstrated in conditions such as pancreatitis (Vincent and Bihari, 1992), heart failure (Levine et al, 1990) and salicylate poisoning (Leatherman and Schmitz, 1991). Thus, sepsis is a clinical manifestation of the host response to various insults, including infection, but also other acute diseases associated

with inflammation. The presence of bacteria in the blood is clearly not a requirement for the diagnosis of sepsis. The term septicaemia should be reserved for the association of demonstrable bacteraemia and the clinical syndrome of sepsis.

The development of a variably distensible and permeable vascular bed is one of the major achievements of mammalian evolution. It allows blood flow to a tissue to be determined by its oxygen requirement and to vary according to external conditions. As a result the cardiovascular system can cope with various demands, such as extremes of temperature and vigorous exercise. In septic shock this system breaks down. Septic shock is sepsis with evidence of inadequate tissue perfusion. Arterial hypotension is an important clinical sign, but is not always present because the disorder primarily involves the microcirculation. While shock describes the clinical syndrome, acute circulatory failure is the underlying pathophysiological process. The presence of tissue hypoxia is reflected by an increase in blood lactate levels. The degree of hypotension, the requirement for vasopressors, and the severity of the lactic acidosis are directly related to outcome (Bakker et al, 1991; Vincent et al, 1992).

As defined above, septic shock is characterised by circulatory failure. This usually involves systemic vasodilatation, pulmonary vasoconstriction, and increased vascular permeability. This haemodynamic state may be induced by administration of endotoxin. Serum from septic patients has been

shown to dilate catecholamine-precontracted aortic smooth muscle (Hollenberg et al, 1992), and the haemodynamic response to infused *Escherichia coli* in baboons is abolished by pretreatment with anti-TNF antibody (Tracey et al, 1987). This, and other evidence (Ayres, 1992), implicates cytokines in the causation of the vasculopathy of septic shock. Indeed, progressively severe vascular paralysis may be the defining characteristic of sepsis, while increasing concentrations of lactate, interleukin-1 [IL-1], TNF, and other cytokines may define the transition from sepsis to septic shock and the development of multiple organ failure.

Septic shock is associated with dysfunction of major organs such as the heart, lungs, liver and kidneys. The extent of organ dysfunction can vary, and it is difficult to define the point at which organ failure begins. There are two distinct pathways by which organ dysfunction may develop. In primary organ dysfunction, there is a direct insult to the organ. Examples include aspiration of gastric contents into the lungs, and the effect of rhabdomyolysis on the kidneys. This direct insult causes an inflammatory response that is localised to the affected organ. Secondary organ dysfunction, such as adult respiratory distress syndrome [ARDS], results from a systemic inflammatory response, and commonly occurs in septic shock. Multiple organ dysfunction in critically ill patients is usually secondary, and results from a complex and generalised disorder of vasomotor tone and permeability of the vascular bed.

The term "sepsis syndrome" (Bone et al, 1991), indicates the

association of sepsis due to any cause, and altered organ perfusion or function. Since sepsis itself is no more than a clinical syndrome, and no longer presupposes infection, sepsis syndrome would seem to be redundant. It has been argued that the term "systemic inflammatory response syndrome" be used instead of sepsis (Bone et al, 1992). Others have argued that the introduction of further terms will only lead to more confusion (Vincent and Bihari, 1992).

In conclusion, whilst many terms are still used to describe the same syndromes, a degree of consensus is developing. Sepsis is a clinical syndrome, which occurs in response to various insults, and reflects the systemic immune mediated response. Septic shock is sepsis associated with evidence of circulatory failure, indicated by increasing blood lactate concentration with or without arterial hypotension. Multiple organ failure indicates failure of more than one organ which is not due to localised pathology of the organ, but is mediated by a systemic vasculopathy. These are the terms which will be used in this thesis. Quantitative definitions will be given where necessary.

(2) Adrenocortical Function During Critical Illness?

The biochemistry and physiology of adrenocortical function are discussed in Chapter 3. The background to the study of adrenocortical function during critical illness is discussed here. As long ago 1893, Zappert reported that the number of circulating eosinophils and lymphocytes fell during severe

infections. Hills (1948) showed that these changes were dependent on a functioning adrenal cortex. Studies of the steroid or eosinophil response to surgery demonstrated a consistent postoperative increase in steroid levels or fall in eosinophil levels which lasted about 48 hours in uncomplicated cases, (Sandberg et al, 1954; Moore, 1957) and much longer in patients developing serious complications (Venning, 1945). With the advent of reliable cortisol assays, similar findings were reported during episodes of severe illness, (Melby and Spink, 1958; Cornil et al, 1968) and the possibility that the adrenocortical response may be inadequate in some patients was raised (Swingle et al, 1942; Sibbald et al, 1976).

It is important to distinguish between impairment of adrenocortical function and insufficiency of adrenocortical hormones. The former may be common during critical illness whereas the latter is considered to be rare. For example, widespread haemorrhage within the adrenal cortex can occur as a complication of septicaemia due to *Neisseria meningitidis* (Melby, 1961; Migeon et al, 1967) and other organisms (Melby and Spink, 1958). Although the adrenal cortex is damaged and its function is likely to be impaired to a degree, absolute adrenocortical insufficiency is unusual (Xarli et al, 1978).

Impairment

The prevalence of impaired adrenocortical function during critical illness is unknown. Adrenal ischaemia and necrosis

are common in pathological studies of experimental shock in animals (Lopes de Faria et al, 1979; Kajihara et al, 1983), and post mortem studies following circulatory shock (Mack et al, 1969) and severe burns (Arai et al, 1976) in man. However, the extent to which this is associated with impairment of adrenocortical function is unclear.

Hypotension and shock associated with severe illness might impair adrenocortical function. Renal blood flow is maintained with greater efficiency than adrenal blood flow in animal models of both haemorrhagic and endotoxic shock (Akiyama et al, 1987). Cortisol secretion is profoundly influenced by adrenal perfusion (Urquhart, 1965; L'Age et al, 1970). Studies of haemorrhagic shock in dogs show that adrenocortical sensitivity to ACTH is not impaired, but that as blood pressure falls, adrenal blood flow is reduced and cortisol secretion diminishes (Mack and Egdahl, 1970). Reperfusion of the adrenal artery markedly increases cortisol secretion. Moreover, whilst all dogs increase cortisol secretion initially in response to haemorrhagic shock, increased secretion persists only in those dogs in which adrenal blood flow is maintained (Hume and Nelson, 1954). In man, positive pressure ventilation, which is frequently required during critical illness, has been shown to reduce adrenal blood flow by 25% (Manny et al, 1979). A number of drugs commonly used in critical illness, including dopamine (Morra et al, 1990), benzodiazepines (Nilsson, 1990) and ketoconazole (Couch et al, 1987; Best et al, 1987) reduce cortisol secretion.

Septic shock due to Gram-negative infection appears to be most frequently associated with impairment of adrenocortical function. Plasma cortisol levels are lower in Gram-negative infection than in Gram-positive infection [Schein et al, 1990]. Severe toxemia due to typhoid infection is associated with lower cortisol levels than non-septic critically ill controls (Khosla et al, 1989), and steroid replacement may sometimes be indicated (Hoffman et al, 1984). Pathological studies have demonstrated severe destructive changes in the adrenal cortex following endotoxic shock [Bardiakhchian and Kirichenko, 1986]. Injection of E. Coli lipopolysaccharide (Catalano et al, 1984; Garcia et al, 1990) or Gram-negative septic shock plasma (Keri et al, 1981) impairs ACTH-induced steroidogenesis in rats.

There are isolated reports of patients suffering from septic shock who do not respond to ACTH during their illness but regain normal function on recovery (Jacobs and Nabarro, 1969; Journey et al, 1987; Varma et al, 1990). Validation of ACTH stimulation tests has been carried out in healthy individuals (Wood et al, 1965; Crowley et al, 1991) or in out-patient populations (Lindholm and Kellet, 1987; Stewart et al, 1988). In health, the cortisol increment following ACTH stimulation is independent of the basal cortisol concentration (Kukreja and Williams et al, 1981; May and Carey, 1985; Dickstein et al, 1991), but interpretation of tests performed in critically ill patients with high baseline cortisol concentrations is difficult. However, there are reports of impaired responses to ACTH in critically ill

patients with low cortisol concentrations. Two patients investigated by Journey et al (1981) had stimulated cortisol concentrations below 400 nmol/L. Both were suffering from sepsis, were given cortisol replacement therapy, and had a normal response to a repeat ACTH stimulation test on recovery. Sibbald et al (1977) identified five patients suffering from septic shock with baseline cortisol concentrations of less than 400 nmol/L, and cortisol increments of less than 100 nmol/L. Interestingly, the only patient to survive was treated with cortisol.

In summary, there is good evidence that adrenal haemorrhage, necrosis and hypoperfusion occur in animal models of endotoxic and haemorrhagic shock and to some extent during critical illness in man. However, there is only anecdotal evidence that this results in impairment of adrenocortical function in man.

Insufficiency

Defining adrenal insufficiency during critical illness is problematic. Increased cortisol concentrations are required during illness. Patients with Addison's disease require increased cortisol replacement, and animals succumb rapidly to experimentally induced sepsis following adrenalectomy, but survive if given steroid replacement (Hinshaw et al, 1985; Bertini et al, 1988). The adrenocortical response to illness is necessary for survival. Since cortisol concentrations increase in response to illness, and the capacity to increase cortisol concentration is necessary for survival,

it follows that the level of adrenocortical function below which is insufficient must also increase. The criteria used to define adrenocortical insufficiency in otherwise healthy patients cannot be applied in critical illness. This was demonstrated very clearly by observations made by Finlay and McKee (1982). In an ICU population, they found a high prevalence of cortisol concentrations within the normal range for health. This observation coincided with a sudden increase in mortality on their ICU which appeared to be confined to those patients with low cortisol concentrations and a relatively small cortisol response to ACTH stimulation tests. It transpired that these patients had been given etomidate (Watt and Ledingham, 1984), a new anaesthetic agent which was subsequently shown to impair adrenocortical function (Fellows et al, 1983). On the basis of their baseline cortisol concentrations and response to ACTH, the majority of these patients did not have absolute adrenocortical insufficiency as defined for otherwise healthy individuals, but their adrenocortical function was sufficiently impaired to increase mortality. In other words, their adrenocortical function was insufficient relative to the severity of their illness. Indeed, cortisol replacement in physiological dosage reduced mortality (McKee and Finlay, 1983).

Swingle et al (1942) first suggested that refractory shock might be partly due to adrenal failure. More recently, similarities between decompensated Addison's disease and sepsis, namely fever, hypotension, high-output cardiac

failure, and low systemic vascular resistance, have been pointed out (Melby et al, 1988, Dorin and Kearns, 1988). It was suggested some years ago that administration of hydrocortisone in moderate dosage reduced mortality (Swingle et al, 1944; Knapp and Howard, 1957), although evidence of adrenocortical failure prior to death could not be demonstrated by others. Jennings (1951 and 1952) followed the circulating eosinophil counts of 50 severely ill patients, all of whom died. In 47, the eosinophil count fell to zero or near zero prior to death, suggesting a strong adrenocortical reaction. Sandberg et al (1956) studied 17-hydroxycorticosteroids in dying patients, and found elevated levels in the great majority. Done et al (1958) investigated 64 severely ill adults a few minutes after death. Plasma corticosteroids were raised in the majority of cases, although lower levels were found in 11 cases who had suffered prolonged hypotension or apnoea.

Interest subsequently turned to studies of high dose steroid therapy. However, high dose steroids did not reduce mortality in animal models of septic shock (Hinshaw et al, 1981 and 1982; Ottosson et al, 1987 and 1989) or in clinical trials in human septic shock (Schumer et al, 1976; Sprung et al, 1984; VASSCSG, 1987; Luce et al, 1988). Indeed, high dose steroids may increase mortality, possibly due to secondary infection (Bone et al, 1987). High dose steroids were not used treat potential adrenocortical insufficiency, but to prevent potentially damaging inflammatory cascades. The doses of steroid used, usually 1g methylprednisolone daily,

were hugely supraphysiological and were likely to have impaired adrenocortical responsiveness to ACTH for several days after administration (Weiskopf et al, 1985; Streck and Lockwood, 1979).

The realisation that the development of septic shock is mediated by cytokines, the production of which is suppressed by steroids, has increased interest in the adrenocortical response. Uncontrolled production of cytokines is associated with fatal outcome in critical illness (Waage et al, 1987; Girardin et al, 1988; Marks et al, 1990; Calandra et al, 1991), there is anecdotal evidence that low dose steroid replacement may be beneficial in septic shock. Park and Raggatt (1989) reported haemodynamic and clinical improvement, following hydrocortisone replacement (20mg tid), in 2 patients suffering from septic shock who had plasma cortisol levels of around 400 nmol/L with only small increments following standard ACTH stimulation tests. There are numerous similar reports of apparent benefit in small numbers of patients with relatively low plasma cortisol concentrations or poor responses to ACTH stimulation tests, but in whom there was no absolute adrenocortical insufficiency (Jacobs and Nabarro, 1969; Hubay et al, 1975; Sibbald et al, 1977; Journey et al, 1987; Varma et al, 1990; Voerman et al, 1990). However, little can be concluded from these anecdotal reports.

In summary, there is no evidence that absolute adrenocortical insufficiency is common during critical illness. However, the optimum level of adrenocortical function during

severe illness is difficult to define. If a certain degree of apparent impairment of adrenocortical function was shown to be associated with a higher than expected mortality, then randomised clinical trials of steroid replacement would be worthwhile.

3) Thyroid Function During Critical Illness?

The biochemistry and physiology of thyroid function during health are discussed in Chapter 5. The changes in thyroid hormone concentrations during illness are well documented, and have been termed the "Sick Euthyroid Syndrome". The main diagnostic problem is to distinguish the very ill patient in whom the low total T4 concentration is due to illness from the sick patient with hypothyroidism. The Sick Euthyroid Syndrome is divided into two main subtypes (Chopra et al, 1983):

The Low T3 Syndrome

The low T3 syndrome is characterised by a subnormal serum total T3 and a normal serum total T4. This syndrome occurs in many situations, including liver disease (Hepner and Chopra, 1979), infection (Wartofsky et al, 1977), renal failure (Kaptein et al, 1981; Hardy et al, 1989), ischaemic heart disease (Wiersinga et al, 1981; McAlpine and Cobbe, 1988), burns (Becker et al, 1982), starvation (Portnay et al, 1974) and following surgery (Chu et al, 1991; Kobayashi et al, 1991). Low T3 concentrations are found in 70% of general medical patients in hospital (Kaplan et al, 1982), and virtually all intensive care patients (Slag et al, 1981;

Wehman et al, 1985). The syndrome may also occur following administration of certain drugs, including dexamethasone, amiodarone and propranolol (Cavalieri and Pitt-Rivers, 1981).

The concentration of T3 may be very low, and is often below assay sensitivity. Serum free T4 levels and the FTI are usually normal, but concentrations of free T3 are low. In normal subjects 20% of T3 production stems from thyroidal secretion and 80% from peripheral deiodination of T4. Thyroidal production of T3 is normal during illness (Lim et al, 1977) and the metabolic clearance rate of T3 is little changed from normal (Van der Heyden et al, 1988; Kaptein et al, 1986). Reverse T3 concentrations are markedly increased during illness. This results from increased production and diminished metabolic clearance (Chopra et al, 1976; Kaptein et al, 1982). Peripheral deiodination of T4 to T3 is reduced and deiodination to rT3 is increased. The cause of the altered 5'-deiodinase activity is unknown. Hagenfeldt et al (1979) and others have observed that increases in plasma cortisol precede the alterations in T3 and rT3 concentration, suggesting a causal relationship. However, Brandt et al (1976) showed that the changes in T3 levels following surgery were identical irrespective of whether the patient had undergone general, in which case cortisol concentrations increase, or epidural anaesthesia, in which case cortisol concentrations remain normal. Moreover, the low T3 syndrome occurs in starvation which is not associated with increased cortisol concentrations. Others has suggested that reduced

availability of cytosolic cofactors for 5'-deiodinase, such as NADPH or glutathione, might alter the peripheral T4 metabolism (Kaplan, 1979; Yamada et al, 1982).

The Low T4 Syndrome

The low T4 syndrome is seen in severely ill patients with a variety of systemic illnesses. The T4 concentration is reduced, but the TSH concentration is usually normal. T4 production and secretion by the thyroid gland are normal or only slightly decreased (Kaptein et al, 1982). The concentration of thyroid-binding globulin (TBG) is normal (Chopra and Smith, 1975; Slag et al, 1981) or slightly decreased (Talwar et al, 1976). The concentrations of albumin and thyroid-binding prealbumin (TBPA) are decreased during illness (Helenius and Liewendahl, 1979), but even complete loss of these binding proteins would only increase the free T4 fraction by 30% (Silberman et al, 1988). Since the free T4 fraction is greater than this in many critically ill patients, other factors must be present (Chopra and Smith, 1975).

The thyroid hormone binding capacity of TBG is reduced during illness (Chopra et al, 1979; Woeber and Maddux, 1981) and may be responsible for much of the change in thyroid hormone levels. Free T4 levels are high during illness, whereas the free thyroxine index (FTI) is variable but often low (Woeber and Maddux, 1981). It is argued that the disparate free T4 and FTI results are due to the presence of a thyroid hormone-binding inhibitor that functions well under conditions of equilibrium dialysis, but not under those of

T3 resin uptake used in the calculation of FTI (Kaptein et al. 1981; Vermaak et al, 1983; Surks et al, 1988). The presence in the serum of severely ill patients of such a binding inhibitor has been demonstrated (Chopra et al, 1979; Oppenheimer et al, 1982). The inhibitor is thought to be a substance released from damaged tissue during illness, but the exact nature of the substance or substances remains unclear. There is evidence that certain free fatty acids may be responsible (Mendel et al, 1986; Chopra et al, 1986) but this is not universally accepted (Shifferdecker et al, 1990), and other factors have been suggested (Mendel et al, 1991; Ramaker and Wood, 1990).

The reduced protein binding of T4 leads to accelerated degradation (Gregerman and Solomon, 1967) and increased free hormone concentration. The extent of the fall in T4 concentration correlates with severity of illness and mortality (Slag et al, 1981; Werner, 1981; Philips et al, 1984; Song et al, 1991). Detailed analysis of free T4 levels is difficult due to marked discrepancies between different assays in severe illness (Kaptein et al, 1981; Slag et al, 1981).

There is conflicting evidence regarding TSH concentration in severe illness. Studies performed using early TSH RIAs suggested that TSH levels were normal (Mclarty et al, 1975; Slag et al, 1981; Kaptein et al, 1982; Kaplan et al, 1982), and occasionally high on recovery (Bacci et al, 1982; Hamblin et al, 1986). More recent work with sensitive TSH assays has shown that a proportion of patients have subnormal TSH concentrations (Boles et al, 1987). However, most

studies reporting low TSH levels (Wehman et al, 1985; Arem and Deppe, 1990) included patients receiving treatment with steroids and dopamine which inhibit TSH secretion. The situation is further complicated by the finding that a significant minority of normal healthy subjects have subnormal TSH levels measured by sensitive assays (Chosich et al, 1989; Arem et al, 1990;). The nocturnal surge in TSH secretion is lost in severe illness (Romijn and Wiersinga, 1990), and raised TSH levels in myxoedema fall to normal during illness and rise on recovery (Hooper, 1976). The TSH response to TRH is reduced in severely ill patients (Talwar et al, 1976; Heinen et al, 1981), although the evidence is again confounded by treatment with steroids and dopamine. Maturlo et al (1980) induced a fall in T4 concentration by administration of iodide to a group of ill patients. The basal or TRH-stimulated TSH levels increased in only half the cases, suggesting that the TSH response to a hypothyroid state may be lost in certain patients.

Cytokines may be partly responsible for changes in thyroid function during illness. Injection of IL-1 beta (Dubuis et al, 1988) and TNF alpha (Ozawa et al, 1988) to rats leads to a fall in TSH, T4 and T3 concentrations in rats. The fall in TSH occurs after the fall in thyroid hormone concentrations, suggesting a direct effect of the cytokines on the thyroid gland or hormone metabolism. Treatment of rats with Il-1 impairs the T3 and T4 responses to TSH (Fujii et al, 1989), and decreases pituitary TSH content (Enomoto et al, 1990). TNF alpha has similar effects (Pang et al, 1989), and both

IL-1 and TNF inhibit thyroid cell growth in culture and thyroid hormone release from cultured cells (Sato et al, 1990; Kraiem et al, 1990).

The combination of reduced serum T3 and T4 concentrations found during critical illness indicate a poor prognosis (Heinen et al, 1981; Slag et al, 1981, Vierhapper et al, 1982). However, in general patients are considered to be euthyroid (Faber et al, 1987) with normal or depressed TSH concentrations (Arem and Deppe, 1990). There is some evidence of hypothyroidism at tissue level, estimated using angiotensin converting enzyme activity (Brent et al, 1984; Smallridge et al, 1985) or erythrocyte sodium/potassium adenosine triphosphatase activity (Dasmahapatra et al, 1985). Low tissue concentrations of T3 have also been found (Reichlin et al, 1973). Some authors suggest treatment either with T3 (Hesch et al, 1981; Becker et al, 1982) or with a combination of T3 and T4 (DeGroot, 1989), while others suggest that the low T3 state is metabolically protective and "an important beneficial adaption to illness in man" (Utiger, 1980).

Starvation produces changes in thyroid function similar to those found during illness (Burman et al, 1979; Borst et al, 1983). In calorie-deprived patients, the pulse rate decreases and the QKd interval and systolic time interval are prolonged (Meyers et al, 1980). Basal metabolic rate decreases (Huang et al, 1981), and can be restored to normal with T3 replacement (Mince et al, 1980). It has been suggested that starvation leads to an hypothyroid state inten-

ded to limit catabolism (Wartofsky and Burman, 1982).

Is there any evidence that thyroid hormone replacement is beneficial during illness? In a rat model of pneumococcal septicaemia, thyroxine replacement increased mortality and shortened survival compared to controls (Little, 1985). A Japanese study of T3 replacement in a canine model of haemorrhagic shock (Shigematsu and Shatney et al, 1988), demonstrated dramatic haemodynamic improvements in the treated group, and an impressive reduction in mortality in treated animals (1/13 deaths vs 9/10 deaths). However, a study of a canine model of myocardial infarction by another Japanese worker (Tanaka et al, 1988) failed to demonstrate any effect of T3 replacement on haemodynamic parameters or mortality. An uncontrolled study of T3 replacement in septic shock in man claimed to show an increase in systolic blood pressure, reduced vasopressor requirements, and improvement in renal function in the treated group (Hesch et al, 1981). There have been only two controlled trials of thyroid hormone replacement during severe illness in man. Becker et al (1982) treated 8 patients with severe burns with 200 mcg nasogastric T3 replacement daily. There was no change in metabolic rate compared with controls receiving no treatment, and mortality (50%) was identical in both groups. Brent et al (1986) treated 11 ICU patients with a daily injection of 1.5 mcg/kg thyroxine. Serum concentrations of total T4 and free T3 concentrations were normalised in the treatment group after 5 days. Mortality (73%) was identical to the control group. Thyroid hormone replacement is clearly

not life saving in severe illness, although a more modest effect on mortality or morbidity cannot be excluded.

The association between low concentrations of thyroid hormones and an increased mortality in critical illness has not been studied in detail. This is of particular relevance to the debate regarding thyroxine replacement during illness. Although mortality among patients with low thyroid hormone levels is high, is it higher than would be expected on the basis of illness severity measured using other parameters? Are low thyroid hormone concentrations really associated with an increased mortality when severity of illness is taken into account? This is one of the main questions examined in this thesis.

(4) Difficulties in The Interpretation of Research in Critically ill Patients

Intensive care patients are an extremely heterogeneous group, with a wide range of underlying pathologies. Management regimes differ for each patient, and seldom remain the same from day to day. Research is therefore difficult and findings are not necessarily reproducible. Measurement of any single parameter is likely to be affected by a number of confounding factors related to pathology, monitoring or treatment. Most published research in intensive care medicine ignores these difficulties. Researchers take the pragmatic view that it is not necessary to disentangle the effects of illness from those of intensive care. It is argued that patients must necessarily be subject to both

groups of influences. However, management differs from unit to unit, and new treatments frequently emerge. If results of research involving ICU patients are to be generalisable, then the confounding effect of treatment must be minimised, although a degree of heterogeneity of patients and conditions must be accepted if any clinical research is to be carried out at all.

Endocrine and metabolic changes accompanying critical illness are likely to be particularly susceptible to confounding by external influences. Such influences can be divided into two groups. There are those which affect the majority of patients equally, such as continuous noise and twenty four hour lighting, and those which affect only a proportion of patients, such as haemodialysis, physiotherapy, and intravenous nutrition. The effects of the former are difficult to measure without elaborate control data. The effects of the latter can be assessed. One of the aims of this thesis was to define conditions in which some of these influences could be minimised.

Research into the endocrine response to critical illness is also hampered by the difficulty in defining what should be regarded as normal in critically ill patients. The definition of normality of a measurement is often arbitrary, and will vary depending on purpose for which the measurement is to be used. For example, a normal response to a standard ACTH stimulation test has been defined by identifying the line of demarcation between the response to ACTH in patients diagnosed by other methods to be suffering from hypoadrenal-

ism and the response of individuals with apparently normal adrenocortical function. This approach, frequently used by endocrinologists to define normality, is quite different from the two standard deviations about the mean of a population used elsewhere. The line of demarcation between normal and abnormal or insufficient endocrine function is unclear in critically ill patients. The dynamic tests of adrenocortical and thyroid function used in this thesis are not used to identify abnormality but simply to define the range of responses expected in a population of severely ill patients.

(5) Ethical Difficulties of Research During Critical illness

It is difficult to draw a line between what is medical practice and what is research. As the Belmont Report (1983) noted, the distinction "is blurred partly because both occur together ... and partly because departures from standard practice are often called experimental when the terms experimental and research are not carefully defined." The Royal College of Physicians (1990) defined medical practice as "an activity undertaken solely with the intention of benefiting an individual patient, where there is a reasonable chance of success." It was further stated that "the progressive modification of methods of investigation and treatment in the light of experience is a normal feature of medical practice and is not to be considered as research." By contrast, "Where an activity involving a patient is undertaken with the prime purpose of testing a hypothesis and permitting conclusions to be drawn in the hope of con-

tributing to general knowledge, this is research."

Medical research may be divided into therapeutic and non-therapeutic. Therapeutic research, such as a clinical trial comparing two established treatments, is relatively easy to justify. Non-therapeutic research, into which category this thesis falls, involves no benefit to the patient, but is simply "a systematic investigation designed to develop or contribute to generalisable knowledge" (Levine, 1979). The distinction is important when considering the issue of informed consent. Informed consent requires that the following conditions be met: the patient involved in a study is given sufficient information in order that he is able to decide whether the proposed research is something in which he would be willing to participate; consent must be voluntarily given; the patient must have the legal capacity to consent to the proposed research. Levine (1988) lists 16 separate elements of informed consent.

In intensive care research there are difficulties with informed consent because patients are frequently unconscious. For the purposes of acute care in this situation, the requirement for informed consent is routinely abandoned under the auspice of beneficence. This practice is justified on the grounds of the legal concept of implied consent which assumes that patients needing immediate medical intervention would want such care in order to avoid death or reduce morbidity (Buchanan and Brock, 1989). It has been suggested that this principle should also apply to therapeutic research in acute care of unconscious patients (Iserson and

Mahowald, 1992). This may or may not be reasonable, but the same principal cannot be applied to non-therapeutic research. The requirement for consent in this situation was considered by the Royal College of Physicians (1990), where it was suggested that a near relative should be informed of the nature of the research and should concur. It was stated that "the legal status of non-therapeutic research involving patients who are incompetent through the severity of their illness is quite uncertain In general, the patient should be told about participation in research later when he recovers sufficiently to comprehend."

Consent by a relative or other surrogate is neither legally nor morally as binding as consent from the patient. It is not certain that surrogate decision-makers always have the best wishes of the patient in mind (Boyle, 1990), and there is a tendency to comply with the wishes of the researchers. Schaffner et al (1988) found that in more than 700 consecutive cases of post-resuscitation coma involved in clinical studies, no relative removed a patient from the research protocol. In this thesis, a proportion of the research was performed soon after patients were admitted to the ICU. At this point in time relatives are frequently extremely distressed about the deterioration in the condition, or sudden onset of illness, in their relative. It seems unnecessarily cruel to compound their anxiety by requesting the right to "experiment" on the patient.

In severely ill patients who are conscious, consent is paradoxically more problematic than in unconscious patients.

Whether severely ill patients really have the capacity to make free and informed decisions has rightly been questioned (Frost, 1975). Studies of consent prior to elective surgery show that patients understand little about the procedure, and cannot remember their informed consent (Robinson and Merav, 1976). Critically ill patients are often suffering great distress, and are unlikely to give any decision adequate consideration, even if they are able to comprehend the issues. There is a danger that patients may be fearful of offending the physician who is seeking consent, by declining to take part in research. They are often aware of their uncertain prognosis, and may be willing to go to considerable lengths to oblige a physician, whom they perceive to have a degree of influence over their fate.

In the case of many of the patients included in this work, the only intervention to which they were subject was the taking of a single sample of blood. This was almost invariably obtained at a time when blood was also being sampled for routine investigations, and accounted for an additional blood loss of only 10ml. A record of routinely collected data was kept outwith the medical records for each patient included in the study. This was securely stored and the format was anonymous. It was felt that in such cases, bearing in mind the difficulties with informed consent in ICU patients outlined above, consent from the patient or relative would not be sought. However, on recovery the patient would be told that some of the results of tests performed during their illness would be used for the purposes of

research.

The situation in patients in whom we wished to perform dynamic tests of endocrine function, requiring the administration of drugs, or in whom repeated blood sampling was necessary, was less clear cut. The tests performed in this study are standard diagnostic tests of proven safety used in everyday clinical practice. The Ethical Committee decided that when a patient was conscious, consent should be obtained, but in the cases of an unconscious patient, dynamic tests could be performed without consent of a relative, the patient being told of the research on recovery.

Chapter 2

Patients, Setting and Assays

- (1) Setting
 - (2) Patients
 - (3) The APACHE II Severity of Disease Classification System
 - (4) Principals of Radioimmunoassay
 - (5) Cortisol Measurement and Validation of Assay
 - (6) Thyrotropin Measurement
 - (7) Thyroid Hormone Measurement
 - (8) Interpretation of Total Hormone Concentrations during illness.
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(1) Setting

All investigations detailed in this thesis were performed on the Intensive Care Unit at South Cleveland Hospital, Middlesbrough. This is an 8 bed ICU, which has the capacity to care for 8 patients requiring ventilation and 24 hour nursing care and up to 6 patients requiring regular haemodialysis. In addition to haemodialysis, the unit has considerable experience of continuous arteriovenous haemofiltration. Continuous cardiac monitoring, in situ transducer measurements of central venous and arterial blood pressure, ventilation pressures, end expiratory carbon dioxide, and transcutaneous oxygen saturation are available at each bed. The ICU is one of two subregional IUCs serving a population of 500,000. The second unit at Middlesbrough General Hospital is dedicated to the care of trauma, neurosurgery and

neurology cases. The study ICU is mainly a medical and general surgical unit. It provides the subregional renal intensive care service, and is referred patients requiring intensive care from five local district general hospitals. Patients on the unit are under the care of a full-time consultant in Intensive Care Medicine, with support from two consultants in Renal Medicine. One senior registrar and two senior house officers are attached to the unit. There is a separate neonatal and paediatric intensive care unit.

(2) Patients

The 260 patients investigated formed 90% of all ICU admissions over a 2 year period. Of the 29 patients excluded, 21 were receiving dopamine on admission, which alters thyroid and pituitary function, 5 were on longterm steroid therapy, and 3 were receiving T4 replacement. The principal diagnoses of patients studied are shown in Table 2.1. The average age of patients studied was 59.6 years [range 14-86] and there was a slight excess of males, 137 vs 123 females.

The mean APACHE II score [see below] of patients studied was 19.8 [SD = 8.6] with a range of 7 - 41. Eighty eight patients died prior to discharge from the unit, giving an overall mortality of 31%. The mean duration from ICU admission to death was 10 days [range, 2 hours - 69 days]. Ninety five patients (37%) were admitted within 24 hours of elective or emergency general surgery and a further 26 patients (10%) were admitted with later postoperative complications. No patient declined consent to any of the tests detailed in

this thesis.

Table 2.1. Principal diagnosis in the 260 patients studied

DIAGNOSIS	PATIENTS [%]
Septicaemia	59 [23]
Abdominal surgery for benign disease	41 [16]
Abdominal surgery for malignant disease	34 [13]
Pelvic surgery	11 [4]
Abdominal aortic aneurysm surgery	19 [7]
Pneumonia	23 [9]
Asthma	19 [7]
Guillain-Barre Syndrome	9 [3]
Multiple trauma	5 [2]
Acute pancreatitis	8 [3]
Eclampsia	3 [1]
Other diagnosis	29 [11]
Total	260 [100]

(3) The APACHE II Severity of Disease Classification System

The APACHE II system (Knaus et al ,1985) is made up of three elements: the acute physiology score; the age score; the chronic health score. The acute physiology score is the sum of the 12 scores obtained from Table 2.2. In this thesis, the term APACHE II score has been used to refer to the acute physiology score alone. The acute physiology score measures physiological derangement which is usually equated with severity of illness.

Table 2.2. The APACHE II severity of disease classification system: the scores given to each of the 12 physiological parameters which make up the acute physiology score.

PHYSIOLOGICAL VARIABLE	HIGH ABNORMAL RANGE				NORMAL	LOW ABNORMAL RANGE			
	+4	+3	+2	+1	0	+1	+2	+3	+4
RECTAL TEMPERATURE (°C)	>41	39-41	-	38.5-39	36-38.4	34-36	32-34	30-32	<30
MEAN ARTERIAL PRESSURE (mmHg)	<160	130-159	110-129	-	70-109	-	50-69	-	<49
RESPIRATORY RATE (ventilated or not)	>50	35-49	-	25-34	12-24	10-11	6-9	-	<5
OXYGENATION (if FIO ₂ >0.5 use A-aDO ₂)	>500	350-499	200-349	-	<200	-	-	-	-
(if FIO ₂ <0.5 use PaO ₂)	-	-	-	-	<70	61-70	-	55-60	<55
ARTERIAL pH	>7.7	7.6-7.7	-	7.5-7.6	7.3-7.5	-	7.2-7.3	7.1-7.2	<7.1
SERUM SODIUM (mmol/L)	>180	160-179	155-159	150-154	130-149	-	120-129	111-119	<110
SERUM POTASSIUM (mmol/L)	>7	6-6.9	-	5.5-6	3.5-5.4	3-3.4	2.5-3	-	<2.5
SERUM CREATININE (mg/100ml) (x2 if acute renal failure)	>3.5	2-3.4	1.5-2	-	0.6-1.4	-	<0.6	-	-
HAEMATOCRIT (%)	>60	-	50-60	46-50	30-46	-	20-30	-	<20
WHITE BLOOD COUNT (1000s/mm ³)	>40	-	20-40	15-20	3-15	-	1-3	-	<1
GLASGOW COMA SCORE (GCS)	points score equal to 15 minus actual GCS								

The APACHE II system has two additional elements [Table 2.3]. The age score allocates additional points purely on the basis of the age of the patient. The chronic health score allocates additional points on the basis of a past history of severe chronic illness. This thesis is primarily concerned with the effects of acute severe illness and so the age points and chronic health points are not used.

Table 2.3. The derivation of the age and the chronic health status elements of the APACHE II Severity of Illness Classification System.

(a) Age Score

AGE (YRS):	<45	45 - 54	55 - 64	65 - 74	>75
POINTS:	0	2	3	5	6

(b) Chronic Health Score

If the patient has a history of severe organ system insufficiency or is immuno-compromised assign points as follows:

- 1) For non-operative or emergency postoperative patients - 5 points;
- 2) For elective postoperative patients - 2 points

Definitions

Organ insufficiency or immuno-compromised state must have been evident prior to this hospital admission and conform to the following criteria.

LIVER: Biopsy proven cirrhosis or portal hypertension, episodes of upper GI bleeding attributed to portal hypertension or prior episodes of hepatic failure/encephalopathy/coma.

CARDIOVASCULAR: New York Heart Association class IV.

RESPIRATORY: Chronic restrictive, obstructive or vascular disease resulting in severe exercise restriction, i.e, unable to climb stairs or perform household duties, or documented chronic hypoxia, hypercapnoea, secondary polycythaemia, severe pulmonary hypertension (>40mmHg), or respirator dependency.

RENAL: Receiving chronic dialysis.

IMMUNO-COMPROMISED: Patient receiving immuno-suppressant drugs, chemotherapy, radiation, long term steroids, or had disease which is sufficiently advanced to suppress resistance to infection eg. leukaemia, AIDS.

(4) The Principals of Radioimmunoassay

Radioimmunoassay [RIA] has a high degree of specificity and relative freedom from interference. The specificity of cortisol RIA methods tends to vary with the antiserum that is used, but when using a specific antiserum, significant interference is only likely to be encountered in patients

being treated with prednisone or prednisolone.

The basis of RIA is the competition between radioactively labelled and unlabelled hormone for a fixed but limiting number of binding sites on antibody molecules. Radioactively labelled hormone is added in excess to all assay tubes. In the absence of any unlabelled hormone, all the antibody binding sites will be occupied by radioactive hormone. If unlabelled hormone is present, it will compete with the radioactive species for the available binding sites, and as the concentration of unlabelled hormone increases, so more labelled hormone will be displaced from the bound fraction. Using standard hormone preparations of known concentration, the binding of radioactively labelled hormone at each concentration of standard can be determined, and a calibration curve constructed. Hormone concentrations from biological test samples are obtained from such a curve by interpolation. When unlabelled hormone is present in large excess, only a few binding sites will be occupied by radioactive hormone making measurement difficult. In this case the assay is repeated with the biological sample diluted with standard containing no hormone.

The procedure for RIA involves several steps. First, antibodies specific for a hormone have to be obtained. A supply of radioactive hormone is required. All tubes in the assay contain the same mass of antibody and the same excess of labelled hormone, that is the number of binding sites is fixed and sufficient label is present to fill all of them. Some tubes contain known amounts of unlabelled hormone

arranged incrementally to give a standard curve.

The biological test sample is added to the tube and left to incubate under specified conditions for a fixed period. At the end of the incubation period labelled hormone bound to antibody must be separated from that remaining free in solution. This separation is the major technical problem because antibody is generally so dilute that no precipitation occurs when the hormone-anti-hormone complex forms.

Production of antisera is the first stage in the development of an RIA. This can be achieved by animal immunisation or by monoclonal antibody techniques. For steroids, a major problem is that the molecule itself is not antigenic. It must be coupled to a larger antigenic molecule to form a structure [hapten] which will be recognised by the animal which is to produce antibodies. Antibodies which react with the hapten will exhibit some cross-reactivity with the steroid. Proteins to which steroids are frequently coupled are bovine serum albumin, ovalbumin, and keyhole limpet haemocyanin.

In animal immunisation techniques, rabbits and sheep are most often used. The hormone is injected in an oil-in-water emulsion with Freund's adjuvant [a mineral oil containing dead tubercular bacilli, which sensitises the immune system in a non-specific way to the presenting antigen]. The antibody is then extracted from blood taken from the animal some weeks or months after injection of antigen.

To produce monoclonal antibodies, specific strains of mice or rat are injected with hormone immunogen. Lymphocytes from the spleens of these animals, which are likely to be produ-

cing antibodies, are fused with appropriate myeloma cells and then grown in culture. The hybrid cells which retain the capacity for antibody secretion from the lymphocytes, and the ability to grow well in culture from the myeloma cells, are selected out and propagated. Anti-hormone can be harvested from the cell culture medium.

Steroid RIAs use either tritium [H^3] or radioactive iodine [I^{125}] as tracers. Tritium has the advantage of a longer half-life, and the fact that it can be bonded directly to the steroid ring structure. Radioactive iodine cannot be bonded directly to the steroid ring structure without significantly changing its conformation. This problem is circumvented by binding I^{125} to a bridging molecule such as histamine or tyramine. In this way the I^{125} produces minimal interference in the hormone-antibody reaction. Despite these problems I^{125} is more popular in steroid RIAs than H^3 because it has a higher specific activity and yields assays that are much cheaper and more robust. Gamma particles are emitted by I^{125} , and these are more easily counted than the beta particles emitted by H^3 .

Separation of antibody-bound and free radioactive label is a limiting factor in the accuracy of many RIAs. Briefly, separation is most commonly achieved using charcoal adsorption, precipitating antibody, or solid-phase antibody techniques. In the charcoal technique, free hormone is adsorbed onto the charcoal leaving antibody-bound hormone in solution. Solid phase techniques utilise anti-IgG antibody bound to microspheres such as sepharose beads, or some other solid

material. Most assays now use some variation of the solid phase antibody technique.

Assay Validation

In the early days of immunoassay, each laboratory had to produce and evaluate all its own reagents, and validate assays based on those reagents. Most hospital clinical chemistry laboratories now use commercial radioimmunoassay kits, of which there are a considerable number. Criteria for the acceptability of kits have been published (Fraser and Wilde, 1986). Kits used in clinical laboratories in the United Kingdom are evaluated by the National External Quality Assessment Scheme [NEQAS]. However, it is recommended that each laboratory validates its own use of a particular assay kit. It is necessary to know the accuracy and precision of an assay if it is to be used in clinical research in relatively small numbers of patients in order that significant changes in hormone concentration can be differentiated from assay variability.

Accuracy is defined as closeness to the "true" value. It can be assessed by analysing a standard for which the concentration of analyte has been determined by a reference method, such as gas chromatography / mass spectroscopy. Standards in human serum are available from NEQAS. Precision is defined as the spread of replicate observations about the mean. It is expressed as a standard deviation [SD] or as the coefficient of variation [CV]. This is defined as $100 \times \text{SD} / \text{mean}$. The precision of immunoassays varies as a function of dose.

It is recommended that an assay is optimised with reference to its "precision profile" (Ekins and Edwards, 1983). This is a plot of the CV against dose which facilitates the selection of maximum precision in the region of the dose-response curve that is of principal interest to the analyst. In a good steroid RIA the CV at the nadir of this curve should be less than 5%, and should be below 10% throughout the working range of the curve.

The precision between assay batches [inter-assay] will not be as good as that within a single batch [intra-assay]. For sequential studies over a period of time it will be necessary to determine the inter-assay CV. While a precision profile approach can be used for this purpose, it is more common to estimate the CV obtained for a number of quality control samples that are analysed by every assay. At least three quality controls should be used and they should contain hormone concentrations at different points within the working range of the standard curve. Ideally, the mean inter-assay CV should always be below 15%, and should aim to be below 10% (Jeffcoate, 1981).

Sensitivity and detection limit of an assay are often quoted. Although the two terms are used interchangeably, they describe different parameters. The sensitivity of an assay is a feature of the precision at zero analyte concentration, whereas the detection limit refers to the lowest concentration of an analyte that may be distinguished statistically from zero.

(5) Cortisol Measurement and Validation of Assay

Blood sampling and storage

All investigations of adrenocortical function in this thesis rely on accurate measurement of cortisol concentration. The conditions required for accurate sampling of plasma cortisol are detailed below:

1] It is recommended that stress to the patient should be avoided. Increases in plasma cortisol concentration occur very rapidly after even mild stress [Ismail, 1981]. Venepuncture may increase plasma cortisol levels, and so sampling from an indwelling venous catheter is recommended.

2] Venous stasis increases the concentration of plasma proteins in blood. Stasis is likely to occur while searching for a vein, following the application of a tourniquet. Since cortisol is mainly protein bound, venous stasis may produce misleadingly high concentrations.

4] Haemolysis should be avoided as this interferes with radioimmunoassay techniques which assay cortisol in plasma without extraction into an organic phase (Cook and Beastall, 1987). In such direct assays, the standards are commonly made up in serum which will contain none of the proteins from red blood cells.

5] Time of sampling is vital to interpretation of results because of the marked circadian rhythm of cortisol secretion.

Storage of blood for cortisol assay is also important. After collection, blood is transferred to a lithium heparin tube

and centrifuged in order to separate the plasma. Plasma can then be stored at 4°C [a standard refrigerator] for up to 72 hours (Ismail, 1981). Storage for longer periods requires the sample to be frozen at - 20°C.

In this study, venous blood was used for all cortisol measurements. As far as possible study blood samples were taken at the same time as samples for routine non-study investigations. In those patients with central venous lines in place (80%), study samples were taken from the line. All infusions via the central line were stopped for 30 seconds prior to sampling and the first 5 ml of blood drawn from the central line was discarded. In the remaining patients samples were taken immediately following insertion of a 20 French gauge "Butterfly" cannula in an antecubital fossa vein. A tourniquet was used. Blood was drawn into a plastic syringe and 5 ml transferred immediately to a 10 ml plastic lithium-heparin tube for cortisol measurement. Samples were stored at 4°C until transfer to the Clinical Chemistry Laboratory, Middlesbrough General Hospital, where the cortisol assay was performed. All assays were performed within 72 hours of sampling.

Cortisol Assay

Cortisol was measured using a commercial radioimmunoassay kit ("CORT-CT", CIS Bioindustries, Gif-Sur-Yvette, France). The antisera is produced by rabbit immunisation and labelled cortisol is bound to I¹²⁵. The anti-cortisol antibody is bound to the inner surface of a polypropylene tube thus

facilitating separation of bound and free cortisol fractions.

The assay has been used by the Department of Clinical Chemistry at M.G.H. on a regular basis for a number of years and the laboratory staff are experienced in the use of the kit, performing the assay on about 50 clinical samples each week. The assay performance specifications are published by the manufacturer and accuracy of the assay is checked regularly against NEQAS standards.

The sensitivity of the assay is reported by the manufacturers to be 6.8 nmol/L. The antiserum used in the assay shows cross-reactivity with prednisolone [28% of the specificity of cortisol] but not with dexamethasone. The cross-reactivities reported with corticosterone and 11-deoxycortisol are 4.3% and 7.9% respectively. The within assay coefficients of variation are reported to be 5.5% at cortisol concentrations of approximately 200 nmol/L, 3.6% at 600 nmol/L and 6.2% at 1500 nmol/L. The inter-assay coefficients of variation are reported to be 7.9% for cortisol concentrations of 200 nmol/L, 6.8% at 600 nmol/L and 7.2% at 1500 nmol/L.

Nearly all the local experience with the assay involved cortisol concentrations below 1000 nmol/L, and the manufacturers performance data does not give information on precision at concentrations greater than 1500 nmol/L. Although the kit contains a high dose standard cortisol sample in order to produce the standard curve, the jump in concentration between the highest two standards is 825 nmol/L to 2750 nmol/L. The difference in expected gamma counts between

these two concentrations is small compared to the differentials at lower concentrations. The concentrations of reagents in RIAs are calculated to give maximum assay precision within the range of concentrations in which the substance to be measured most frequently occurs. For plasma cortisol this range is 100 - 1000 nmol/L. Many ICU patients have plasma cortisol concentrations above 2000 nmol/L, and as a rule, the further from the intended measurement range a sample becomes, the less precise the assay will be. It is possible to dilute a sample with zero standard, but the measurement of sample and dilution volume introduces further potential inaccuracies, and so ideally dilution should be avoided. It was therefore necessary to validate the assay using samples from ICU patients with high cortisol concentrations.

Validation of Cortisol Assay

Intra-assay variation was calculated using 6 samples of plasma taken from six ICU patients. The samples were chosen to reflect the range of cortisol concentrations regularly encountered in ICU patients. Following initial plasma cortisol measurement each sample was divided into 10 portions. Plasma cortisol was measured in each portion from each of the 6 samples, using the same assay kit for all 60 measurements. All remeasurements were performed on the same day by a single technician. In Table 2.4, the intra-assay variation in measurement of plasma cortisol concentration is presented as the coefficient of variation.

Table 2.4 Mean [SD] plasma cortisol concentration and intra-assay coefficient of variation.

SAMPLE	PLASMA CORTISOL CONCENTRATION [nmol/L]		
	MEAN	SD	CV [%]
1	326	16.2	5.0
2	749	24.1	3.2
3	1127	76.0	6.7
4	1783	128.4	7.2
5	2012	158.9	7.9
6	2431	204.0	8.4

The intra-assay coefficient of variation at lower plasma cortisol concentrations was similar to that published by the manufacturers, but increased at concentrations above 1000 nmol/L. A coefficient of variation of 8.4% at a concentration of 2431 nmol/L represents 95% confidence limits for a sample of that concentration of 2023 - 2839 nmol/L. In other words, if 2 samples both measure 2431 nmol/L, it is possible that their true cortisol concentrations differ by over 800 nmol/L. This is likely to be a considerable hindrance to the interpretation of short dynamic tests of adrenocortical function in patients with high basal cortisol levels.

The inter-assay coefficients of variation were not further validated. It is likely that they would be 1 or 2% greater than the intra-assay coefficients at each concentration. Since only a very small number of dynamic tests will involve

the use of more than one assay kit, this error is less important than the within-assay error.

There were some difficulties in interpretation of the results of one assay kit. A series of dexamethasone tests were performed on ICU patients and controls, but are not included in this thesis. Following an oral dose of 2mg dexamethasone in the evening, plasma cortisol was sampled at 0800h the next morning. In the 5 healthy controls, the morning cortisol concentrations ranged from 60 nmol/L to 160 nmol/L. The mean concentration had fallen from 216 nmol/l [SD = 51] prior to the dexamethasone to 107 nmol/l [SD = 31]. However, the post-dexamethasone levels were higher than would be expected. The majority of individuals should have cortisol concentrations of less than 80 nmol/L on the morning of a dexamethasone suppression test.

There are a number of possible interpretations of the apparent failure of cortisol to suppress fully in 4 of the 5 controls. All were young females and may have been taking the oral contraceptive pill which would increase CBG concentrations, although such an effect would be unlikely in itself to account for all of the failure to suppress. Depression is associated with a failure of cortisol to suppress following dexamethasone, but this was not evident in any of the controls. Three of the controls were nursing staff working on the night shift in whom the normal diurnal rhythm of cortisol concentration would have been disrupted. However, this should not have had a major effect on suppression following dexamethasone, although the influence of shift

work on mood may have had an effect. There may have been a problem with the accuracy of the assay at low and low normal concentrations. Although, the laboratory had not had problems previously with low hormone concentrations, it may be relevant that all five tests in controls were performed on the same day, and samples were, therefore, assayed by a single technician using the same assay kit.

The vast majority of cortisol concentrations detailed in this thesis were above 300 nmol/L. Any problem with the assay at low concentrations would not, therefore, be a major problem. However, the difficulty with the dexamethasone suppression tests illustrates the potential difficulty associated with the use of an assay at concentrations out-with the usual range.

Diurnal Variation in Plasma Cortisol Concentration

Diurnal variation in plasma cortisol concentration was studied for two reasons. Firstly, repeated measurement of plasma cortisol concentration over a short period in the same patient will give an impression of the extent of the short term variability in cortisol concentration during critical illness. It is, of course, impossible to judge how much of the variance is due to changes in the true hormone concentration and how much is due to measurement error. However, in terms of validating the cortisol assay for use in dynamic tests of adrenocortical function, an assessment of the background short term variability in cortisol concentration would be very useful. Secondly, although the

diurnal variation in cortisol secretion is known to be blunted during illness, it is not clear whether the rhythm is completely abolished in critically ill patients. This is important with respect to the timing of tests of adrenocortical function.

In order to determine the diurnal variation in plasma cortisol concentration in ICU patients, 10 consecutive admissions to the unit who fulfilled the following criteria were investigated: 1) acute illness of between 2 and 28 days duration; 2) no anaesthetic within the past 24 hours; 3) not receiving opiates, catecholamines, dopamine, steroids or etomidate; 3) APACHE II score greater than 25.

Patients were investigated on a day when no stressful procedures were planned. Blood was taken for plasma cortisol estimation at 2 hourly intervals for 24 hours, starting at 0800h on the study day. The APACHE II score was calculated on data collected during the study day. No patient underwent haemodialysis or haemofiltration during the 24 hour period. Physiotherapy and intravenous fluids were given as clinically indicated.

The mean [SD] APACHE II score of patients studied was 31.7 [2.4]. Six were male, 4 female, and mean [SD] age was 61 [7] years. Data collection was 97% complete. A single cortisol measurement was missing in 2 cases, and 2 measurements were missing in a further case. No consistent pattern of diurnal variation is seen in the individual cases [Fig 2.1]. However, although the mean plasma cortisol concentration remained fairly constant from 10.00h to 20.00h, it did fall

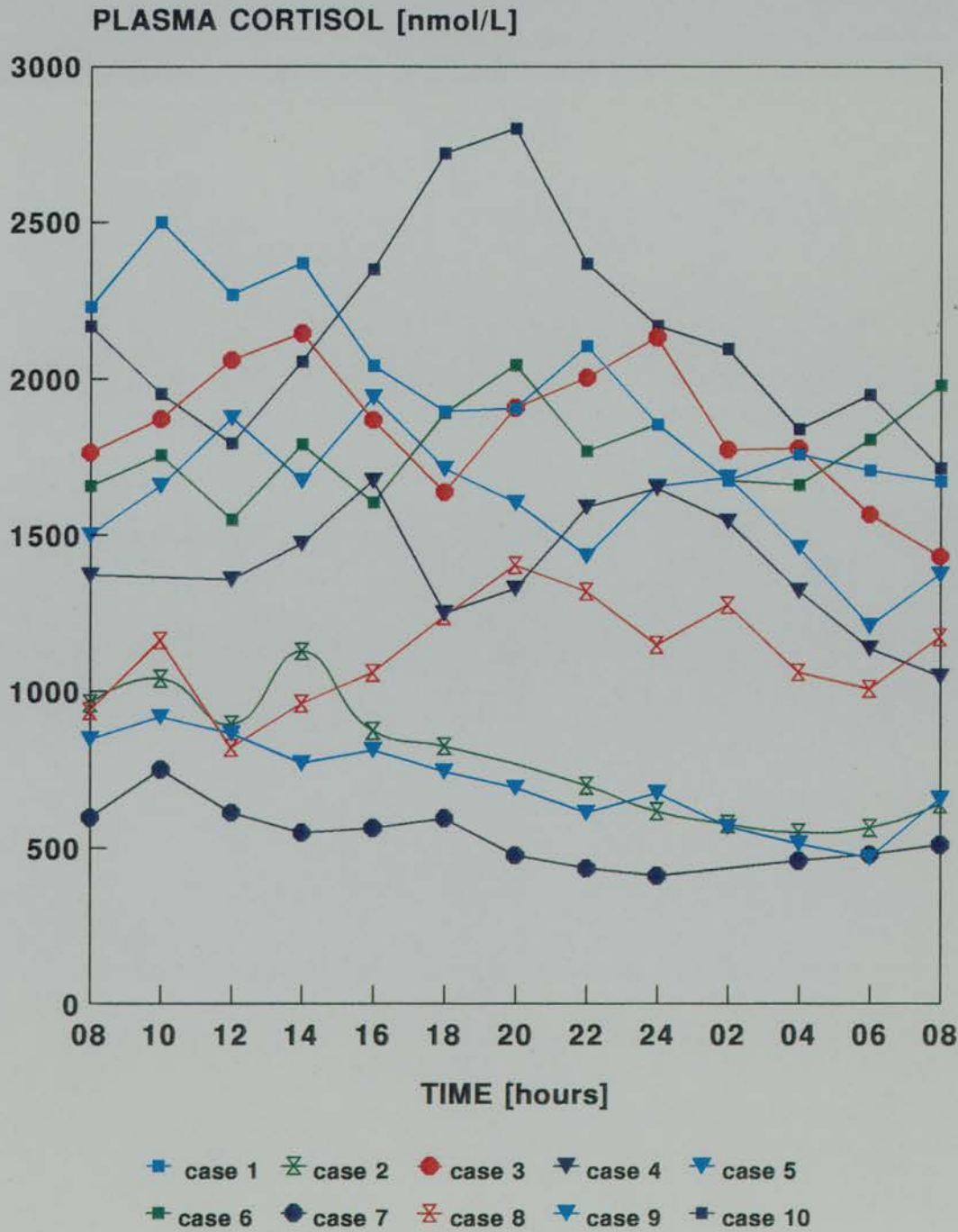
thereafter, reaching a low point at 06.00h. The absolute decrease in plasma cortisol concentration between 20.00h and 06.00h was significant [MD = 281 nmol/L, 99% CI = 119 - 442] as was the trend between those times (regression slope = 0.21, $P < 0.01$).

Table 2.5. Mean [SD] plasma cortisol concentration in 10 patients over 24 hrs

TIME	CORTISOL CONCENTRATION [nmol/L]	
	MEAN	SD
0800	1403	534
1000	1501	552
1200	1429	557
1400	1491	589
1600	1479	577
1800	1428	552
2000	1540	612
2200	1433	634
2400	1417	620
0200	1430	501
0400	1328	484
0600	1188	523
0800	1221	420

Although 5 cases had cortisol concentrations consistently greater than 1500 nmol/L, with the exception of case 10, the 2 hour changes in cortisol concentration were seldom greater than 250 nmol/L [Fig 3.1].

FIG 2.1 CIRCADIAN RHYTHM OF PLASMA CORTISOL CONCENTRATION



MISSING DATA IN CASES 2,4 AND 7.

The first observation of a daily rhythm in cortisol secretion was made in man by Pincus (1943). The normal range of cortisol concentration is 220 - 720 nmol/l at 0800h, and less than 220 nmol/l at midnight (Ismail, 1981). This rhythmicity may be related to light and dark variation, since nocturnal animals show a pattern which is exactly opposite to diurnal animals. Halberg (1959) showed that these patterns do not correspond exactly to 24 hours, and named them circadian rhythms. These rhythms are thought to be induced by an internal "clock" which integrates signals from a number of external stimuli, or zeitgeber, such as light, food and sleep. In critical illness this diurnal change is blunted (Perkoff et al, 1959), and may be absent (Schein et al, 1991). Similar changes occur following major surgery (McIntosh et al, 1981).

There are a number of factors, in addition to sustained stress of critical illness, which might contribute to the loss of circadian rhythm in cortisol concentration. Shifts in the sleep-wake pattern profoundly disturb the corticoid rhythm (Orth et al, 1967). Indeed, sleep appears to be a more powerful synchroniser than light (Krieger et al, 1969). Many ICU patients are sedated and sleep all day. Unsedated patients have little sleep pattern due to continuous noise, monitoring and nursing care. Any consistent influence of sleep on cortisol secretion is likely to be lost. A constant light environment markedly reduces the amplitude of the circadian rhythm of cortisol (Cheifetz et al, 1968; Krieger, 1973), and has been shown in rats to decrease the response

to stress (Paluch et al, 1983). Intensive care units are well lit over the whole 24 hour period. Alterations in pattern of feeding alter adrenocortical rhythmicity (Krieger, 1974; Wilkinson et al, 1979). This is relevant to ICU patients, most of whom are fed by infusion. There are therefore a number of external influences which might alter the circadian rhythm of cortisol secretion.

(6) Thyrotropin Measurement

Venous blood was used for all measurements of thyroid function. Blood was sampled as detailed above for plasma cortisol. Blood was drawn into a plastic syringe and transferred into a lithium-heparin tube. Storage and transfer of samples was the same as that used for cortisol samples.

Thyrotropin was measured using a sensitive immunoradiometric assay (TSH MAIAclone, Serono Diagnostics, Woking, Surrey, UK). This assay uses two high affinity monoclonal antibodies and has a higher sensitivity and specificity than traditional assays (Rattle et al, 1984). The sample to be measured is mixed with the two antibodies. Both antibodies, one attached to ^{125}I and one attached to fluorescein, bind to discrete sites on the TSH molecule. Following incubation, anti-fluorescein antibody attached to a magnetic solid phase is added in excess. The complex thus formed is separated using a magnetic field, and the concentration of antigen is directly proportional to the radioactivity bound to the separation agent, measured using a gamma counter.

The performance characteristics of the assay are reported by

the manufacturers for the range of values expected in this study. No further validation of the assay was considered necessary. The accuracy of the assay has been assessed by recovery experiments performed by adding purified TSH to serum samples. The percentage recovery ranged from 91% to 106% in 15 samples. The intra-assay coefficient of variation did not exceed 3.2% at any point on the standard curve, and the inter-assay variation was consistently below 4%. The detection limit of the assay was between 0.02 and 0.04 mU/L. There was little cross-reactivity with hCG, FSH, or LH. The assay gives equivalent values with serum and plasma samples. Grossly haemolysed or lipaemic samples may interfere with the assay.

(7) Thyroid Hormone Measurement

Total T4 was measured using an unpublished, "in-house" RIA. This is a double antibody, peg accelerated RIA technique. First antibody is obtained from Scottish Antibody Production Unit (SAPU), and ^{125}I -T4 from Amersham International (London, UK). The normal range of T4 using this assay is 60 - 160 nmol/L. The intra-assay coefficient of variation is 5.4%, and the interassay coefficient is 7.2%. T3 was also measured using an unpublished, "in-house" RIA. The method is identical to that used in the T4 assay. The first antibody is obtained from SAPU, and ^{125}I -T3 from Amersham international. The normal range is 1.1 - 3.2 nmol/L, and intra-assay coefficient of variation is 9.8%. Neither of the thyroid hormone assays were further validated.

(8) Interpretation of Total Hormone Concentrations during Illness.

In this thesis, only total concentrations of thyroid hormones and cortisol are measured. This limits the interpretation of the data, but does not undermine the aims of the thesis. The difficulties of interpretation of total hormone concentration and measurement of free hormone levels are discussed below.

Free Cortisol and Cortisol Binding

The concentration of CBG is reported to be increased during severe illness (Barnton and Passingham, 1981). However, the very high cortisol concentrations found in many critically ill patients would be expected to saturate CBG binding. The excess cortisol would bind more weakly to albumin, thereby increasing free cortisol concentration. Reliable free plasma cortisol assay is not widely available, but what data there is suggests that free cortisol does increase during illness (Murray, 1967; Barton and Passingham, 1981). There is little published work on cortisol metabolism and excretion during severe illness.

It was not possible to measure free plasma cortisol in this study. Urinary free cortisol could have been measured. The small amounts of unmetabolised cortisol excreted in the urine are measured by RIA. Urinary free cortisol excretion is dependent upon the output of cortisol by the adrenal cortex during the period of urine collection, and the plasma free cortisol concentration. It is a useful measurement in

the diagnosis of adrenal hyperfunction. It is, however, dependent upon renal perfusion and functional capacity, and is liable to give low values in renal failure, and high values in states of increased renal blood flow. This can be partly corrected for by expressing urinary free cortisol as a ratio with urinary creatinine, but altered renal physiology still presents problems. Over half of patients on a medical ICU have some impairment of renal function. The proportion is higher in the severely ill patients who are the subjects of much of this study. Many patients receive diuretics and infusions of dopamine and adrenaline which further alter renal physiology. It was therefore felt that results of urine free cortisol measurements would be difficult to interpret. Measurement of CBG in the absence of an index of plasma free cortisol would not be particularly helpful.

The lack of information about plasma free cortisol concentration precludes any comment about the likelihood of tissue insufficiency. It also makes an understanding of any changes in the control of the hypothalamic-pituitary-adrenal axis during illness difficult. However, these were not the aims of this thesis. The relationships between total plasma cortisol and severity of illness and mortality can be usefully interpreted and the response of the adrenal cortex to ACTH may lead to meaningful conclusions.

Free Thyroid Hormones and Thyroxine Binding Globulin

As discussed in Chapter 1, the equilibrium between free and



protein-bound circulating thyroid hormones is altered during illness. Total thyroid hormone concentrations are no longer a useful surrogate measure of free thyroid hormone levels. For the same reason, measurement of the concentration of thyroid hormone binding proteins would not allow useful conclusions to be drawn about free thyroid hormone availability. If the concentration of binding proteins increased during illness, the presence of binding inhibitors would still be likely to result in increased concentrations of free thyroid hormones. In fact, TBG concentrations are normal or slightly reduced during illness (Chopra and Smith, 1975; Helenius and Liewendahl, 1979; Slag et al, 1981).

The need for serum free T4 measurement led to the development of the equilibrium dialysis technique (Sterling and Brenner, 1966). This is technically demanding and so the T3 uptake test evolved as an estimate of the unoccupied T4 binding sites on serum TBG (Braverman et al, 1967). However, the free T4 index (FT4I) has poor reliability in severely ill patients in whom artefactually low FT4I values are frequently encountered (Woeber and Maddux, 1981). In recent years, the assay of free thyroid hormones has become possible (Jackson and Ekins, 1986), and many clinical laboratories now measure the free hormone directly rather than measure total hormone. However, there is disagreement between the direct and indirect methods of measuring free thyroid hormone during critical illness (Kaptein et al, 1981; Vermaak et al, 1983; Surks et al, 1988). Melmed et al (1982) reported a reduced FT4 by equilibrium dialysis in 43%

of ICU patients. Commercial FT4 assays on the same patients were subnormal in between 30% and 100% depending upon the kit. Wang et al (1985) reported low FT4 values in ICU patients using ultrafiltration and RIA methods. However, Surks et al (1988) found that FT4 was normal in 30 ICU patients using both ultrafiltration and equilibrium dialysis. Finally, Kaplan et al (1982) and Krenning et al (1987) reported elevated FT4 values in 23% and 54% respectively of severely ill patients.

Given the major difficulty in measurement and interpretation of free thyroid hormone levels during illness, only total T3 and T4 levels were measured in this thesis. This limits any comment on the possibility of hypothyroidism in the patients studied and undermines any detailed understanding of the altered control of the hypothalamic-pituitary-thyroid axis. However, these were not the aims of the studies detailed in this thesis.

Chapter 3

Historical Background, Structure, Physiology and Investigation of Adrenocortical Function

- (1) Historical Background
 - (2) Anatomy, Biochemistry and Physiology of Adrenocortical Function
 - (3) The Concept of "Stress" and Adrenocortical Function
 - (4) Investigation of Adrenocortical Function
-

(1) Historical Background

The first detailed description of the adrenal glands, and their recognition as an organ, was made in 1563 by Bartholomaeus Eustachius Sanctoserinus in his treatise *De Glandulis Quae Renibus Incumbunt*. In 1849, Thomas Addison presented a paper to the South London Medical Society, entitled *On Anaemia: Disease of the Suprarenal Capsules*. In 1855 he published a monograph, entitled *On the Constitutional and Local Effects of Disease of the Supra-Renal Capsules*, in which he gave the first description of chronic adrenocortical insufficiency. Early studies of adrenal pathophysiology were conducted by men whose names are more often linked with other fields of medicine. The year after Addison published his monograph, Brown-Sequard discovered in animal experiments that the adrenal glands are necessary for life. However, it was not until 1896 that Sir William Osler recognised the efficacy of orally administered adrenal extracts in the treatment of Addison's disease.

The 20th Century has seen the elucidation of much of the biochemistry of steroid hormones, and the mechanisms of regulation and control of adrenocortical function. In 1926, Smith demonstrated that hypophysectomy led to atrophy of the adrenals, and Evans succeeded in preventing this atrophy by the administration of pituitary extracts. In 1942, Li and Sayers isolated ACTH. In 1946, Seyle described his "General Adaption

Theory" of adrenal function, and in 1948 Hench and collaborators detected the anti-inflammatory effect of cortisone.

The development of radioimmunoassay during the late 1950s and the 1960s coincided with a period of great advance in the chemistry of peptide hormones. By 1962, Hofmann, Li, and Schwyzer had described the amino acid sequence of ACTH, and in 1966, Schwyzer and Seiber succeeded in synthesising the hormone. In the past decade corticotropin releasing hormone [CRH] has been characterised and synthesised (Vale et al, 1981), and many of the factors which control its secretion, such as opiates and neural mechanisms, have been recognised.

(2) Anatomy, Biochemistry and Physiology of Adrenocortical Function

The functioning of the adrenal cortex is complex, and much of the detail is beyond the scope of this thesis. However, in order to facilitate discussion in later chapters, the basic anatomy, histology, biochemistry, and physiology of the adrenal cortex and control of the hypothalamic-pituitary-adrenal [H-P-A] axis will be discussed. The adrenal medulla is an embryologically, histologically, biochemically, and functionally different organ to the cortex, and will not be discussed.

The adrenal glands are paired organs lying in the retroperitoneal space, anteromedial to the upper pole of the kidneys. In the adult human, the size of the adrenal glands is about 1/30th of that of the kidneys, weighing between 3 and 5g, and measuring 5.0 cm in length, 2.5 cm in width and only about 0.6 cm in thickness. The cortex develops from cells of the coelomic epithelium and is therefore of mesodermal origin, while the medulla is derived from the neural ectoderm. During illness the adrenal glands hypertrophy. Glands examined at autopsy after prolonged illness have a mean weight of 6 g, and may weigh up to 9 g.

The blood supply to the adrenal gland derives from numerous small arteries arising from the inferior phrenic artery, aorta, and renal arteries. The blood passes through the cortex and then medulla, the resulting high intramedullary cortisol levels being necessary for adrenaline synthesis. Venous blood empties into a single vein, which enters the inferior vena cava on the right, and the renal vein on the left.

The adrenal cortex constitutes approximately 80% of the gland, and is divided into three distinct histological layers. The zona glomerulosa comprises the outer 5% of the cortex, and is responsible for aldosterone secretion. The zona fasciculata forms 70% of the cortex, and its cell

columns are continuous with the cell groups of the zona glomerulosa. The zona fasciculata is responsible for glucocorticoid and androgen secretion. The zona reticularis comprises the inner 25% of the adrenal cortex and also produces glucocorticoids and androgens.

Among approximately 70 different steroids produced by the adrenal cortex, cortisol, corticosterone, aldosterone, and androgenic steroids are the main secretory products. During a 24 hour period the adult human adrenal cortex secretes approximately 10 to 30 mg of cortisol, 2 to 4 mg of corticosterone, and 100 to 200 mcg of aldosterone. The most prominent androgens secreted by the cortex are androstenedione and dehydroepiandrosterone.

Cholesterol is probably the precursor of all adrenal steroid hormones. The cortex is second only to neural tissue in its stores of cholesterol esters, which it derives mainly from plasma low density lipoproteins. Cells are able to synthesise cholesterol de novo, but produce less than 20% of cholesterol used for hormone synthesis. The rate-limiting step in steroid hormone synthesis involves cleavage of the side chain from the 27 carbon atom cholesterol molecule to form the 21-carbon steroid skeleton, pregnenolone. The enzyme responsible is a desmolase complex containing a reaction-specific cytochrome P450.

Pregnenolone undergoes hydroxyoxidation and double-bond isomerisation in the mitochondria, and 3 dehydrogenation reactions in the endoplasmic reticulum to form cortisol. Corticosterone and aldosterone are formed by different combinations of hydroxylation reactions, but contain the same basic carbon skeleton as cortisol.

Cortisol diffuses rapidly into the circulation without significant storage in the adrenal cortex. Cortisol represents approximately 80% of total 17-hydroxysteroids in the blood. Steroid hormones are secreted into the circulation as free hormones, where they bind to plasma proteins. Only free hormone is biologically active, and the amount of hormone available to the tissues is determined by the equilibrium between free and bound fractions. Between 3% and 10% of cortisol is in the free state. Of the remainder, 90% is bound to corticosteroid-binding globulin [CBG] and 10% is bound to albumin. CBG is a glycoprotein synthesised in the liver, containing one steroid binding site per molecule. Albumin is present in the plasma in much higher concentration than CBG, but binds cortisol only very weakly.

The binding of corticosteroids to proteins such as CBG provides a reser-

voir for the hormones in plasma, serves to dampen acute changes in hormone levels, and protects the hormone against metabolic degradation. During stress when cortisol levels rise dramatically, the CBG binding sites become saturated. Albumin sites do not, and under these circumstances free cortisol concentrations rise. Most studies of adrenocortical function measure total cortisol levels, and so factors which alter binding protein levels are important. High CBG levels are found in pregnancy and in women taking the contraceptive pill, due to oestrogens. Low levels are seen in liver disease and states of protein loss. The concentration of CBG is reported to be increased in severe illness (Murray, 1967; Barnton and Passingham, 1981).

The liver and the kidney are the principal organs involved in clearing steroid hormones from the circulation. Unconjugated steroids that are filtered by the kidney are largely reabsorbed. Hepatic metabolism leading to conjugation of steroids, mainly as glucuronides, serves two functions: a decrease in biological activity of the hormones, and an increase in their water solubility, thus enabling excretion by the kidney. The plasma half life of cortisol is 60 - 100 minutes.

The adrenal steroids influence a wide variety of biochemical and physiological phenomena, and are essential for life. Steroid and thyroid hormones share the same mechanism of action. These hormones diffuse through the target cell membrane and interact with cytosolic hormone-specific receptor proteins. There are 5000 to 100,000 glucocorticoid receptors per cell. The hormone-receptor complex binds to specific DNA sequences of the hormone responsive gene, leading to increased or decreased gene transcription. The altered protein production is responsible for the hormonal response seen in that particular tissue.

There are six classes of steroid receptors, corresponding to the six known biological activities of the steroid hormones: glucocorticoid, mineralocorticoid, progestin, oestrogen, androgen, and vitamin D. A given steroid hormone will exert an effects in tissues by its by interacting with the specific receptor that recognises that steroid. Cortisol binds to two receptors, the glucocorticoid receptor and the mineralocorticoid receptor. Steroids may exert more than one effect in a given tissue if they interact with more than one receptor in that tissue. The relative affinity of a steroid for each receptor is a major determinant of the bioactivity of that hormone. Cortisol is a potent glucocorticoid with definite, but much weaker, effects on electrolyte and water meta-

bolism. Aldosterone exerts its prime actions on electrolyte and water metabolism, with less than one third the potency of cortisol for glucocorticoid receptors. In some tissues, cortisol exerts very little mineralocorticoid activity due to its inactivation by a receptor-associated enzyme.

A given steroid may also exert diverse biological effects in different tissues. The diversity of hormonal responses is determined by the different genes that are regulated by the hormone in different tissues. Glucocorticoids, for example, exhibit primarily metabolic effects in the liver and primarily anti-inflammatory effects in lymphoid tissue. A large number of genes can be regulated by steroid hormones. It should also be noted that steroids also exert effects not mediated by classical intracellular receptors, but possibly by actions in the cell membrane. Since this thesis is primarily concerned with the glucocorticoid function of the adrenal cortex, the actions of other steroid hormones will not be discussed.

Cortisol interacts in a permissive fashion with other hormones, including insulin, glucagon, catecholamines, and growth hormone, to achieve full homeostasis. Excess cortisol increases hepatic glycogen and glucose production and decreases glucose uptake and utilisation in peripheral tissues, thereby tending to cause hyperglycaemia. Cortisol deficiency has exactly the opposite effect. Glucocorticoids increase free fatty acid levels by enhancing lipolysis, decreasing cellular glucose uptake, and decreasing glycerol production which is necessary for the reesterification of fatty acids. Glucocorticoids exert a generally catabolic effect on protein metabolism, with proteolysis in fat, skeletal muscle, bone, lymphoid, and connective tissue. This increases amino acid substrates which can be used in gluconeogenesis. Cardiac muscle and the diaphragm are almost entirely spared from this catabolic effect.

The physiological role of glucocorticoids in immune regulation is not well understood. At high concentrations they inhibit most immunologic and inflammatory responses. Glucocorticoids stabilise lysosomes and inhibit prostaglandin synthesis and the actions of bradykinin. They block histamine and slow-reacting substance of anaphylaxis, and inhibit production of cytokines. These actions inhibit vasoactive substances and diminish the inflammatory process. Glucocorticoids cause lymphocytopenia with a relative T-cell depletion, monocytopenia, and eosinopenia, while increasing polymorphonuclear cell release. They tend to inhibit cell-

mediated immunity, but have little effect on antibody production. Glucocorticoids have a positive inotropic effect on the heart, increasing left ventricular work index (Davies et al, 1981). Moreover, they have a permissive effect on the actions of adrenaline and noradrenaline. In glucocorticoid deficiency, decreased cardiac output and shock may develop (Dorin and Kearnns, 1988; Melby et al, 1988), and steroid excess causes hypertension (Nasjletti et al, 1984). Glucocorticoids also influence renal physiology. They increase glomerular filtration rate and renal blood flow, and lead to an increase in free water clearance (Noda et al, 1983). Glucocorticoids also have effects on bone, connective tissue, gastrointestinal tract, blood constituents, and the central nervous system, which are not of major relevance to this thesis and are detailed elsewhere (Pescovitz et al, 1990).

The actions of glucocorticoid hormones are seen most clearly in "stress" situations, when glucocorticoid secretion can increase almost ten fold. This is believed to enhance survival through increased cardiac output, increased sensitivity to the pressor effects of catecholamines, increased work capacity of skeletal muscles, and increased capacity to mobilise energy through gluconeogenesis, proteolysis, and lipolysis.

Regulation and control of cortisol concentration

Plasma cortisol concentration is dependent upon cortisol secretion by the adrenal cortex, the degree of protein binding, and the rates of metabolism and excretion of the hormone. In health, short term changes in concentration are mainly determined by changes in cortisol secretion. Binding, metabolism and excretion remain relatively constant in comparison. Cortisol secretion is regulated by the hypothalamic-pituitary-adrenal axis. Cortisol secretion is directly dependent on the plasma concentration of ACTH. ACTH is a 39 amino acid peptide produced by the anterior pituitary gland from a larger precursor known as pro-opiomelanocortin [POMC]. ACTH interacts with membrane-bound receptors on adrenocortical cells which activate adenylate cyclase and increase intracellular cyclic-AMP [cAMP]. This, in turn, activates a protein kinase which ultimately leads to conversion of cholesterol to pregnenolone. ACTH also enhances the later steps in steroidogenesis, increasing uptake of cholesterol from the plasma lipoproteins, and promoting protein synthesis in the cortex, thus maintaining the size of the adrenal gland. It appears that increased cholesterol uptake from plasma is the main point of

regulation by ACTH. ACTH has a number of extra-adrenal effects, the best known of which is its lipolytic action on adipose tissue.

Cortisol secretion is therefore indirectly controlled by those factors which determine ACTH secretion. In common with the other anterior pituitary hormones, ACTH secretion is principally dependent on factors released by the hypothalamus. The anterior pituitary gland or adenophysis has no direct neural connection to the hypothalamus or other parts of the brain. Factors produced in the hypothalamus which are responsible for release or inhibition of release of hormones by the adenophysis reach their target cells via the hypophyseal-portal venous system. Transfer of neuroregulators from hypothalamic neurones to the pituitary blood supply takes place in an anatomically specialised region of the ventral hypothalamus known as the median eminence or tuberoinfundibular region. The tuberoinfundibular neurones projecting to this region terminate in close proximity to capillary walls within the median eminence, which drain into the venous system, that in turn supplies the sinusoids of the adenophysis.

The principal hypothalamic factor responsible for control of ACTH secretion is corticotropin-releasing hormone [CRH]. CRH is among the more widely distributed neuroendocrine peptides and is also found outside the CNS eg. in peripheral leukocytes. However, the paraventricular nucleus of the hypothalamus [PVN] has been convincingly implicated as the source of adenophysisotropic CRH (Makara et al, 1981). Specifically, the majority of CRH-immunoreactive neurones are located in the dorsal aspect of the medial parvocellular subdivision of this nucleus (Swanson et al, 1983). This is supported by the finding that CRH mRNA and peptide levels in the parvocellular neurosecretory zone of the PVN, and peptide content of the median eminence and hypophyseal portal plasma are markedly upregulated by corticosteroid withdrawal (Keller-Wood and Dallman, 1984; Swanson and Simmons, 1989) in keeping with the negative feedback of glucocorticoids on the hypothalamus. However, in addition to the parvocellular cells projecting to the median eminence, CRH-staining neurones are also found in other parts of the PVN, where they project to other neural sites including the brainstem and, in particular, the locus ceruleus.

Although CRH is the primary and obligatory hypothalamic ACTH secretagogue, there are a number of other intrinsically weaker secretagogues, including arginine vasopressin [AVP], oxytocin and adrenaline (Plotsky,

1991). Vasopressin is considered to be the major secondary regulator of ACTH release. These and other peptides are produced by the hypothalamic CRH neurones and are capable of interacting with CRH to stimulate ACTH secretion. Moreover, other hypothalamic cell types, notably magnocellular neurosecretory neurones may produce peptides which modify ACTH secretion (Holmes et al, 1986). Opioid peptides, including fragments of POMC, endorphins, and enkephalins also inhibit CRH and vasopressin release. The clinical relevance of these endogenous opioids is unclear. Of direct relevance to intensive care patients, pharmacological doses of exogenous opioids have been shown to suppress the H-P-A axis and reduce cortisol concentration, in contrast to naloxone which increases plasma cortisol levels (Ferri et al, 1980). Pharmacological doses of catecholamines inhibit ACTH release (Weiner and Ganong, 1978), but intravenous adrenaline has been shown to markedly increase plasma cortisol levels (Tilders et al, 1985).

The H-P-A axis responds to a wide variety of stresses and a number of disparate stress paradigms are capable of modifying CRH mRNA levels as well as CRH release (Lightman and Young, 1988; Herman et al, 1989). The H-P-A axis achieves a balance between the positive drive by various forms of stress and the negative feedback regulation by corticosteroids. Cortisol and synthetic adrenocortical hormones exert an inhibitory effect on hypothalamic CRH secretion, as well as on ACTH secretion by anterior pituitary corticotrophs, which parallels their glucocorticoid and anti-inflammatory activity. Thus prednisolone is five times more inhibitory than cortisol and dexamethasone forty times more inhibitory. The administration of high doses of glucocorticoids leads to gradual functional impairment and atrophy of the adrenal cortex, which is prevented by concomitant administration of ACTH. In man, ACTH secretion is completely suppressed by daily administration of 75 mg of cortisone acetate, and adrenal atrophy develops rapidly.

In keeping with the great diversity of stimuli capable of inducing a stress response, the hypophysisotropic zone of the PVN is known to receive a rich afferent innervation. The afferent sources may be grouped into four classes (Sawchenko and Swanson, 1985). Firstly a series of mainly catecholaminergic pathways relay visceral sensory information from the nucleus of the tractus solitarius. These convey inputs from the thoracic and abdominal viscera via the vagus and glossopharyngeal nerves. Secondly, nearly all cell groups in the hypothalamus send pro-

jections to the hypophysiotropic zone of the PVN, allowing integration of the H-P-A axis with other neuroendocrine, autonomic and behavioural regulatory mechanisms. Thirdly, a number of cell groups in the limbic region of the telencephalon, including portions of the amygdaloid and hippocampal complex, are thought to exert tonic inhibitory influences on neuroendocrine functioning. More specifically, these areas are thought to be potential sites for corticosteroid negative feedback on CRH secretion (Kovacs and Makara, 1988). Finally, a series of interconnected cell groups constituting the lamina terminalis (the rostral margin of the third ventricle), which lie outside the blood-brain barrier, may allow blood-borne factors, such as angiotensin II, to influence the H-P-A axis (Gross, 1987). Emotional and other conscious stimuli are thought to traverse vaguely defined corticolimbic pathways funnelling to the PVN via the bed nucleus of the stria terminalis, the lateral and medial septum and the preoptic area (Gray, 1991).

In summary, the hypothalamus receives several stress related inputs and responds by altering secretion of CRH and a number of other intrinsically weaker ACTH secretagogues. The exact mechanisms by which the hypothalamus synthesises the various inputs are beyond the scope of this thesis and for the most part remain to be determined.

The influence of cytokines on the hypothalamic-pituitary-adrenal axis

The specific interaction of cytokines with the H-P-A axis has been the subject of much recent study. The febrile activity of bacterial and other exogenous pyrogens was known to be mediated by an endogenous pyrogen, which is now considered to be predominantly IL-1. Besedovsky et al (1986) first reported that systemic administration of recombinant IL-1 into mice caused a marked increase in plasma ACTH and corticosterone levels. This work has been confirmed by several others using IL-1, and a number of other cytokines, and it is now evident that cytokines activate the H-P-A axis and mediate, at least in part, the response of the axis to a variety of noxious insults.

Injection of IL-1 causes a prompt rise in plasma ACTH, with the peak appearing at 30 minutes after intravenous injection (Uehara et al, 1987) and 2 hours after intraperitoneal injection (Besedovsky et al, 1986). Intracerebroventricular injection is more potent (Katsura et al, 1988) suggesting that the site of action of IL-1 is in the central nervous system. Intravenous injection of interleukin-6 [IL-6] and TNF-alpha have

a similar though smaller effect on plasma ACTH in rats (Naito et al, 1988). The effect of IL-1, IL-6 and TNF- α on ACTH is abolished by pre-treatment with CRH-antisera (Salposky et al, 1987). Interleukin-2 [IL-2] also has an ACTH-stimulating effect, but acts more slowly, reaching a peak at 4 hours in man, and is unaffected by pre-treatment with CRH-antisera (Lotze et al, 1985), suggesting a site of action other than the hypothalamus. The ACTH-stimulating activity of cytokines can be dissociated from the pyrogenic activity (Naito et al, 1990), suggesting that fever itself is not the factor causing activation of the H-P-A axis. Cytokines are large molecules and should not, in theory, cross the blood-brain barrier. Their site of action within the central nervous system is not clear, but the action may be mediated by prostaglandin release.

In-vitro studies have shown a stimulatory effect of IL-1 on murine and human pituitary corticotrophs (Malarkay and Zvara, 1989), but the effect follows a latent period of over 6 hours suggesting stimulation of production from the POMC gene rather than any effect on ACTH release. IL-2 and IL-6 also appear to have a similar effect (Fukata et al, 1989). TNF- α appears to have no effect on pituitary corticotrophs in-vitro (Kehrer et al, 1988). The physiological implications of such in-vitro experiments are unclear.

Cytokines may act directly on the adrenal cortex, bypassing hypothalamic-pituitary control. IL-1, IL-2, IL-6, and TNF- α increase corticosterone release from dispersed rat adrenal cells (Tominaga et al, 1991). This effect of IL-1 has been confirmed in-vivo in rats (Roh et al, 1987), but the effect was abolished by indomethacin, suggesting mediation by prostaglandins.

There is evidence that a variety of cytokines activate the H-P-A axis at all levels. IL-1 appears to be the most important, and this is confirmed by the observation that IL-1 receptor antibody blocks the ACTH response to lipopolysaccharide (River et al, 1989). The central nervous system mediated effect is rapid and appears to depend upon CRH release, whereas stimulation of ACTH production by corticotrophs and cortisol release by the adrenal cortex is slower and likely to be due to increased biosynthesis of ACTH and cortisol.

Glucocorticoids inhibit CRH release induced by IL-1 (Cambronero et al, 1989), and inhibit the production of IL-1 by peritoneal macrophages. High IL-1 levels in endotoxic shock are reduced by dexamethasone treat-

ment (Staruch and Wood, 1985). Therefore, there seems to be a complex feedback system. Glucocorticoids exert negative feedback on the pituitary gland and hypothalamus, and also inhibit the H-P-A activation induced by cytokines. To what extent cytokines influence the H-P-A axis in health is unclear, but the very high concentrations of cytokines found in critical illness and sepsis (Michie et al, 1989; Calandra et al, 1991; Fisher et al, 1993) may alter the normal control of the H-P-A axis.

(3) The Concept of "Stress" and Adrenocortical Function

*"Stress is certainly one of the most grandly
imprecise terms in the lexicon of science"*

(Ganong 1963)

The development of the stress concept mirrors the evolution of biological and medical thinking. Claude Bernard's ideas regarding regulation of the milieu interieur included the notion that the organism is capable of counteracting external stimuli which would threaten the maintenance of the internal balance. Cannon (1929) introduced the concept of homeostasis as a closed system of processes which defend the dynamic equilibrium against external forces. It was Seyle (1936) who coined the term "stress" to describe these external forces. Seyle developed his theory of the "General Adaption Syndrome" after finding, in animal experiments, that injection of a crude ovarian extract caused the same response as the injection of any other toxic substance: adrenal hypertrophy, atrophy of the thymus and gastric ulceration. He developed the concept that the body reacts in the same way to any nonspecific noxious agent or tissue damage. It was soon realised that this response was due in major part to activation of adrenocortical secretion.

The concept of the General Adaption Syndrome includes three stages, alarm reaction, resistance, and exhaustion. Cannon proposed that the initial alarm reaction was an adrenomedullary response mediated by the sympathetic nervous system. This results in arousal and a number of physiological changes including tachycardia, glycogen breakdown and lypolysis, providing the energy for the fight or flight reaction. This is complemented by the adrenocortical reaction, starting a few minutes

later, which serves to limit the consequences of the primary reflex by suppressing tissue reactions to damage and the immune reaction to antigens, and leads to stimulation of protein breakdown and carbohydrate formation.

The mechanism by which release of ACTH was induced by stress was unclear. Long (1947) observed that adrenaline was a powerful stimulus for ACTH secretion, and proposed that the adrenomedullary alarm reaction directly induced ACTH secretion. However, it was soon found that removal of the adrenal medulla did not prevent the adrenocortical stress response (Gordon, 1950; Vogt, 1951). Fortier (1951) was first to subdivide stress stimuli, and proposed that "neural" stimuli, such as pain and fear, required intact neural connections to the hypothalamus, whereas "systemic" stimuli, such as tissue damage or toxic agents might stimulate the pituitary gland directly. Work with hypothalamic lesions again suggested different pathways subserving neurogenic and systemic stimuli. Smelik (1959) found that posterior hypothalamic lesions inhibited the adrenal response to painful and emotional stimuli, but enhanced the response to traumatic or systemic stimuli.

In the 1960s there was growing conviction that release of hypothalamic CRF was the final common pathway for pituitary ACTH release, the hypothalamic cells being reached by both the circulation and neural connections. The hypothalamic deafferentation technique, introduced by Halasz and Pupp (1965), helped to confirm this. A number of stimuli, including anoxia (Feldman et al, 1970), anaesthesia (Vermes et al, 1973) and endotoxin (Makara et al, 1970), appeared to be capable of releasing ACTH after complete neural isolation of the hypothalamus. The ACTH response to painful stimulation of hind limbs in animal experiments is abolished by peripheral nerve or spinal cord section (Greer et al, 1970). The hypothalamic connections subserving the effects of visual and auditory stimuli on ACTH release have also been defined (Feldman et al, 1972).

Although it is now accepted that the majority of stresses induce the adrenocortical response via their effect on the hypothalamus, there is evidence that certain factors act directly on the pituitary gland. *Escherichia coli* endotoxin has been shown to stimulate ACTH secretion after pituitary stalk section (Makara et al, 1971), and a number of cytokines, including IL-1 (Fukata et al, 1989), IL-2 (Smith et al, 1989) and IL-6 (Fukata et al, 1989), stimulate ACTH release from pituitary cell suspensions in vitro.

Whilst, the mechanisms underlying Seyle's General Adaption Theory are now becoming clear, it is increasingly recognised that very minor physical and emotional stimuli also increase cortisol secretion. It is difficult, if not impossible, to differentiate between "stress" and everyday life, and it is therefore preferable to consider the adrenocortical system as a servomechanism which continuously follows the changing demands of the environment, rather than a system intended to respond to specific insults.

(4) Investigation of Adrenocortical Function

The majority of reviews of the investigation of adrenocortical function are confined to the diagnosis of adrenal hyperfunction or hypofunction in otherwise healthy individuals (Muller, 1986; Stewart et al, 1988; Clayton, 1989). There is no published work concerning the validation of standard adrenocortical function tests in intensive care patients. Indeed, the standard approaches to testing adrenocortical function may be inappropriate in critically ill patients with high cortisol concentrations. Despite the obvious difficulties, some workers have applied the standard normal range of plasma cortisol concentration to severely ill patients. For example, Span et al (1992), in a study of 159 ICU patients, concluded that because all patients had basal cortisol concentrations of greater than 250 nmol/l, there was no hypoadrenalism. Strictly speaking, this conclusion would not be valid in healthy individuals, and may be quite wrong in critically ill patients. Many ICU patients have plasma cortisol levels over ten times normal (Sainsbury et al, 1981; Journey et al, 1987; Schein et al, 1990), and an ability to significantly increase cortisol concentration in response to acute illness is necessary for survival (Sibbald et al, 1977; Hinshaw et al, 1985). The increase in cortisol concentration, and the degree of adrenocortical responsiveness necessary, will depend on the type and severity of the illness, and so a normal range for "illness" is inappropriate. The standard adrenocortical function tests used in this thesis are detailed below. The normal ranges for results in healthy populations are given, but should not be applied in critically ill patients. One of the aims of this work is to define normal ranges for tests of adrenocortical function during critical illness.

Measurement of plasma cortisol was discussed in Chapter 2. It is pos-

sible to measure ACTH using RIA techniques. However, ACTH is unstable in whole blood and plasma and also adsorbs strongly onto glass surfaces. Rapid centrifugation and transfer to the laboratory are necessary. It is the definitive test in differentiating between primary and secondary adrenal failure, when very clear differences in ACTH concentration will be found. In the setting of normal or mildly abnormal adrenocortical function single ACTH measurements give little information. ACTH has been measured in critical illness, but did not yield much useful information (Vaughan et al, 1982; Barton et al, 1987). ACTH concentration does not correlate with plasma cortisol concentration (Vaughan et al, 1982). This is, however, not unexpected in view of the episodic nature of ACTH secretion.

Dynamic Tests of Adrenocortical Function

Dynamic endocrine tests provide additional information to that obtained from measurement of single hormones or of trophic-target gland hormone pairs. Such tests are based on either the stimulation or the suppression of endogenous hormone production. Stimulation tests are utilised most often when hypofunction of an endocrine organ is suspected, and are designed to assess the reserve capacity of the organ to form and secrete a hormone. The trophic hormone is usually an hypothalamic or pituitary hormone.

The Standard ACTH Stimulation Test

There are many protocols for ACTH stimulation tests, but each aims to assess the adrenocortical response to synthetic ACTH. These tests are safer and at least as informative as the insulin tolerance test, and have therefore replaced it as the standard tests of adrenocortical reserve. The standard test in North America is the 8 hour intravenous ACTH test, where 25 IU of ACTH is given as a constant infusion over 8 hours and multiple blood samples are taken for cortisol measurement. In the UK, the short Synacthen test or standard ACTH stimulation test, is most widely used as the initial screening test of adrenocortical function. It is more convenient than the 8 hour test and equally informative (Lindholm et al, 1987).

The standard ACTH stimulation test was developed in the 1960s (Ney et al, 1963; Wood et al, 1965; Grieg et al, 1966). The synthetic 24 amino acid polypeptide ["Synacthen", Ciba] used in the test has equivalent

activity to natural corticotropin (Landon et al, 1964). Corticotropin stimulation testing had been described previously, but earlier workers, using ACTH derived from animal pituitary extracts, had used indirect measures of the glucocorticoid response such as eosinophil count and urinary sodium/potassium ratio (Prunty, 1964). Wood et al (1965) described a rapid test which involved measuring plasma cortisol by fluorimetric assay immediately before and 30 minutes after an intramuscular injection of 250 mcg Synacthen. They demonstrated the ability of the test to differentiate between normal controls, patients receiving steroids, and patients with Addison's disease. This work has been repeated and the test altered and further validated by numerous workers (Speckart et al, 1971; Nelson and Tindall, 1978; Lindholm and Kehlet, 1987). The most frequently used variation now involves the intravenous administration of 250 mcg Synacthen, with measurement of plasma cortisol at 30 and 60 minutes thereafter. This method has been shown to produce a cortisol response which correlates very closely with the response to a standard insulin hypoglycaemia test (Kehlet et al, 1976; Stewart et al, 1988), and which is independent of age and sex (Jensen et al, 1988), basal cortisol concentration and time of day (Dickstein et al, 1991). Comparisons of this standard ACTH stimulation test with the metyrapone test indicate that the test reliably assesses integral H-P-A function (Kehlet et al, 1976).

There is disagreement as to how a normal cortisol response to the standard ACTH stimulation test should be defined (Wood et al, 1965; Musa and Dowling, 1967; Greig et al, 1969; May and Carey, 1985). Various diagnostic criteria, based on the basal cortisol concentration (Greig et al, 1986), the stimulated cortisol concentration (Dluhy et al, 1974), or the difference between them (Greig et al, 1967), have been proposed. There is some evidence that these three values may be independent (Kukreja and Williams, 1981; May and Carey, 1985). Although recent editions of the standard textbooks of endocrinology suggest that the cortisol increment is used (Bondy, 1985; Baxter and Tyrrell, 1987), most recent workers conclude that the peak cortisol concentration is the best criterion with which to define a normal response to a standard ACTH stimulation test (Dickstein et al, 1991; Lindholm and Kehlet, 1987). A useful compromise is a peak plasma cortisol concentration of greater than 500 nmol/L and/or an increment in cortisol concentration of greater than 200nmol/L at 30 minutes (Crowley et al, 1991). All the above workers have defined

the normal response to a standard ACTH stimulation test by identifying the line of demarcation between the response to ACTH in patients diagnosed by other methods to be suffering from hypoadrenalism and the response of individuals with apparently normal adrenocortical function. As discussed in Chapter 1, this approach is not entirely appropriate to investigation in critical illness.

The optimum time to sample blood for cortisol following injection of 250 mcg ACTH appears to be between 15 and 30 minutes (Crowley et al, 1991; Dickstein et al, 1990). It has been suggested that in order to observe the peak cortisol response sampling should be carried out at 5 minute intervals for the first 30 minutes following injection of ACTH (Crowley et al, 1991), but this is expensive and there is little reduction in cortisol concentration between the peak and the 30 minutes sample (Leclercq et al, 1972).

Safety of Intravenous ACTH

Intravenous administration of Synacthen appears to be safe. Earlier use of corticotropin from animal pituitary extracts was associated with a significant risk of allergic reaction (Wood et al, 1965). This may have been due to the different amino acid sequence in human corticotropin or to impurities. The corticotropin content of the extract was difficult to quantify and early tests were occasionally complicated by steroid related side-effects such as cardiac failure. Synacthen has an identical amino acid sequence to the first 24 amino acids of the N-terminal end of human ACTH, and should not, therefore, cause any significant allergic reaction. Hypersensitivity reactions and, in one instance, death (Muller et al, 1982) have been reported following the use of Synacthen. This may be related to the markedly supraphysiological dose which is administered in the standard test.

The Low Dose ACTH Stimulation Test

The standard ACTH stimulation test uses a dose of ACTH sufficiently large to achieve maximal adrenocortical secretory capacity. It is therefore a measure of adrenocortical reserve rather than adrenocortical sensitivity to ACTH. A normal cortisol response to 250 mcg Synacthen does not necessarily indicate normal adrenal sensitivity to ACTH. Indeed, a normal response may be seen in steroid-treated patients who subsequently fail to respond adequately to insulin-induced hypoglycaemia

(Cunningham et al, 1983). It can be argued that the use of a pharmacological dose of ACTH is excessive and contributes to confusion in the interpretation of results.

Recent work has confirmed observations by Landon et al (1967) that the adrenal cortex responds to very low "physiological" doses of ACTH. This has led to the development and standardisation of low dose ACTH stimulation tests (Graybeal and Fang, 1985; Crowley et al, 1991; Dickstein et al, 1991). Doses of Synacthen as low as 0.5 mcg (Crowley et al, 1991) and 1.0 mcg (Dickstein et al, 1991) produced a maximal cortisol response by 30 minutes. A dose of 0.09 mcg ACTH produces a cortisol response greater than 200 nmol at 15 mins in some patients (Crowley et al, 1991). In other words, the standard dose of Synacthen may be over 500 times greater than is necessary to maximally stimulate the adrenal cortex. Some patients on long-term low dose steroid treatment have a normal response to 250 mcg Synacthen, but a reduced response, compared with controls, to 1.0 mcg (Dickstein et al, 1991), suggesting reduced adrenocortical sensitivity to ACTH. More work is required to fully validate low dose ACTH stimulation tests, but they may be able to detect subtle alterations in sensitivity of the adrenal cortex to ACTH, not apparent on standard dose testing. The rise in plasma cortisol after low doses of ACTH is of much shorter duration and returns to baseline values in all subjects by 90 mins (Crowley et al, 1991). It seems possible, therefore, that a low dose of ACTH will facilitate release of immediately available cortisol, while a pharmacological dose of 250 mcg, used in a standard ACTH stimulation test, probably induces further synthesis or mobilisation from other pools to sustain the rise.

The Depot ACTH Stimulation Test

If a patient suspected of having adrenocortical insufficiency has an inadequate response to a standard ACTH stimulation test, then further investigation is required. The next stage is a depot ACTH stimulation test, in which the cortisol response to 1000 mcg of an ACTH/zinc phosphate intramuscular depot injection is measured. Samples are taken for cortisol measurement at hourly intervals for 5 hours after the injection. If the plasma cortisol level increases by more than 100% then primary adrenocortical insufficiency can be ruled out [Ismail et al, 1981]. The basal level usually doubles within the first hour and then rises more slowly. The expected values in health are as follows: 1 hour,

600-1250 nmol/l; 2 hours, 750-1500 nmol/l; 3 hours, 800-1550 nmol/l; 4 hours, 950-1650 nmol/l; 5 hours, 1000-1800 nmol/l. An initial normal rise followed by a rapid and sustained fall suggests a limited adrenocortical reserve capacity, and indicates abnormal adrenal function. There have been no studies of depot ACTH stimulation tests in severe illness.

Adrenal atrophy due to long-term steroid therapy or pituitary failure may result in an abnormal depot ACTH stimulation test. In this situation a three day Synacthen test is required to differentiate these from primary adrenocortical insufficiency. In this test 24 hour urine samples are collected for 5 consecutive days and urinary free cortisol and urinary 17-OHCS measured. On the mornings of days 3 to 5, 1000 mcg intramuscular depot Synacthen is given. In the majority of patients with adrenal atrophy, but not Addison's disease, there is a delayed cortisol response.

Chapter 4

A Study of Adrenocortical Function During Critical Illness

(1) Aims

(2) Methods

- (a) Admission cortisol measurement
- (b) Standard ACTH stimulation testing
- (c) Low dose ACTH stimulation testing
- (d) Depot ACTH stimulation testing
- (e) Serial plasma cortisol measurement
- (f) Repeat ACTH stimulation tests on recovery

(3) Results

(4) Figures

(5) Discussion

(6) Conclusions

(1) Aims

The main aims of the studies detailed in this chapter were as follows:

- 1) To determine the relationship between plasma cortisol concentration and severity of illness, as measured by APACHE II scores during critical illness, and to define normal ranges for plasma cortisol concentration at different levels

of illness severity.

2) To define the range of cortisol responses to standard dynamic tests of adrenocortical function during critical illness.

3) To determine whether or not ICU patients with low concentrations of cortisol, taking into account the severity of illness, or impaired responses to dynamic tests of adrenocortical function have a higher than expected mortality.

(2) Patients and Methods

The background to the tests of adrenocortical function detailed in this chapter was given in Chapter 3. The setting in which the investigations were performed was given in Chapter 2. All dynamic tests of adrenocortical function detailed in this chapter were performed in accordance with the protocol given in Appendix 1. Apache II scores were calculated for each patient on the day of each test.

(a) Admission Plasma Cortisol Measurement

Blood was taken for plasma cortisol estimation in 260 consecutive admissions not receiving steroids or dopamine. Blood was sampled, stored, and assayed as detailed in Chapter 2. All samples were taken within 2 hours of admission. If possible, blood was taken prior to commencement of sedation or inotropes. A note was taken of all medications received in the past 24 hours, and whether or not the patient had received steroids in the past 28 days. APACHE II scores were calculated on data collected during the first 24 hours

of admission. The physicians caring for the patients had access to the results of the cortisol assay.

(b) Standard ACTH Stimulation Tests

Corticotropin stimulation tests were performed in 79 patients. Thirty two of these patients were suffering from septic shock and are dealt with in more detail below. The 47 patients not suffering from septic shock were consecutive admissions to the unit who fulfilled the following criteria: a) acute illness of less than 28 days duration; b) no steroid medication in the past month; c) no history of active tuberculosis or adrenal disease; d) no recent treatment with etomidate. Although these patients were not suffering from septic shock, a proportion showed evidence of secondary organ failure. Patients with clinical evidence of infection or positive blood cultures, but who were not shocked were included.

Of the 47 non-shocked patients investigated 25 were female and 22 male. Mean age was 62 years (range 23 - 84). The primary diagnoses were: abdominal surgery for malignancy (11); abdominal surgery for a non-malignant condition (8); pneumonia or respiratory failure (10); pelvic or urological surgery (5); septicaemia (5); upper gastrointestinal haemorrhage (2); Guillain-Barre syndrome (2); others (6).

Corticotropin stimulation tests were performed on the second morning after admission, unless the patient was undergoing haemodialysis or any other procedure likely to compromise the validity of the test. In these cases the test was per-

formed on the next day. All tests were performed between 0900h and 1200h.

A baseline blood sample was taken and 0.25 mg tetracosactrin ("Synacthen", Ciba) was given as an intravenous bolus in 5ml sterile water. Two further blood samples were taken 30 and 60 minutes after the injection, and plasma cortisol was measured. The cortisol response was defined as the difference between the basal concentration and the higher of the 30 and 60 minute concentrations.

ACTH stimulation tests were performed in 32 patients suffering from septic shock. Patients were investigated if they had a clinical diagnosis of septic shock, fulfilled the criteria stated above for non-shocked patients and the following additional criteria: a) requiring inotropic support in order to maintain mean arterial blood pressure above 80 mm Hg; b) urine output less than 20 ml/hour.

Eighteen men and fourteen women met these criteria. Mean age was 59 years (range 25 - 86). The primary diagnoses were pneumonia (16), peritonitis (11), wound infection (2), pelvic abscess (1), salmonella enteritis (1) and acute pancreatitis (1). In these patients, the test was performed within 48 hours of the onset of shock in patients who developed septic shock following admission. In those patients who were admitted already fulfilling our criteria for septic shock, the test was performed within 48 hours of admission. All tests were performed between 0900h and 1200h. Patients with septic shock received standard treatment. Adrenaline infusions, with or without dobutamine, were used for inotro-

pic support and all patients received low dose dopamine infusions (2mcg/kg/min).

Patients with established acute renal failure were treated with intermittent haemodialysis. At the time of the test 25 of the patients with septic shock and 28 non-shocked patients required respiratory support using mechanical ventilation with or without positive end expiratory pressure (PEEP). The results of the ACTH stimulation tests were available to the clinicians caring for the patients. No patient received steroid treatment.

(c) Low Dose ACTH Stimulation Tests

Low dose ACTH stimulation tests were performed in 10 non-shocked patients, 10 patients suffering from septic shock, and 5 healthy controls. In Patients who had had a standard ACTH stimulation test previously, the low dose test was performed only after at least 24 hours had elapsed.

The controls comprised 2 men and 3 women with a mean age of 27 years (range 19 - 34). The non-shocked cases comprised 6 men and 4 women with a mean age of 56 years (range 34 - 76). The septic shock patients comprised 5 men and 5 women with a mean age of 59 years (range 47 - 69).

The low dose corticotropin stimulation test was performed using 10 mcg tetracosactrin (4% of the standard dose). One vial of tetracosactrin was diluted in 49 ml sterile 0.9% saline giving a concentration of tetracosactrin of 5 mcg/ml. The solution was mixed in a plastic container and 2.0 ml was withdrawn into a plastic syringe. The injection was given

within 10 minutes of the tetracosactrin being diluted. The remaining solution was discarded and a new solution made up for each patient. The low dose test was performed in the same manner as the standard dose test. All tests were performed between 0900h and 1200h.

(d) Depot ACTH Stimulation Tests

Five patients suffering from septic shock who had undergone a standard ACTH stimulation test previously and in whom the higher of the post-ACTH stimulation plasma cortisol levels was less than 1000 nmol/L, and who were still suffering from septic shock, were further investigated with a 5 hour depot ACTH stimulation test. Two women and three men were investigated. Mean age was 63 years (range 54 - 78). The same control cases used for the low dose ACTH stimulation tests were investigated again.

Immediately after the basal blood sample was taken, 1mg of depot tetracosactrin (Ciba Laboratories, UK) was given as an undiluted 1 ml injection into deltoid muscle. Further blood samples were taken for plasma cortisol measurement at 1,2,3,4 and 5 hours after injection. The injection was given at 0900h.

(e) Serial Plasma Cortisol Measurements in Septic Shock

Ten consecutive patients suffering from septic shock were investigated. Following the standard ACTH stimulation test a further blood sample was taken for plasma cortisol measurement at 1800h on the same day. Subsequent samples were taken

at 0900h and 1800h each day until death or ICU discharge. Patients were treated as clinically indicated with no restrictions. Six women and four men were investigated. Mean age was 59 (range 25 - 73).

(f) Repeat Standard ACTH Stimulation Tests on Recovery

Ten patients who had undergone standard ACTH testing whilst suffering from septic shock were investigated following discharge from the ICU. A standard ACTH stimulation test was performed at least 7 days after discharge.

(3) Results

Admission Plasma Cortisol Concentration

An admission plasma cortisol measurement was available in all 260 cases. The mean [SD] plasma cortisol concentration was 1012 [621] nmol/L. The frequency distribution of cortisol concentration is shown in Fig 4.1. Cortisol concentrations were not normally distributed, but were skewed towards higher values. Ninety seven cases had cortisol concentrations greater than 1000 nmol/L. Of these 47 were above 1500 nmol/L and 18 above 2000 nmol/L. Only 92 cases had admission cortisol concentrations below 720 nmol/L, the upper limit of the distribution in health. The median plasma cortisol concentration was 916 nmol/L [range 162 - 3750].

There was a significant correlation between plasma cortisol concentration and APACHE II score [$r = 0.436$, $P < 0.01$, Fig 4.3]. Mean plasma cortisol within 3 APACHE II score ranges [< 16 ; $16-24$; > 24] is shown in Table 4.1.

Table 4.1. Plasma cortisol concentration on admission in 260 patients according to APACHE II score

APACHE SCORE	< 16	16 - 24	> 24
NUMBER OF CASES	89	105	66
CORTISOL [nmol/L]			
MEAN [SD]	760 [302]	994 [640]	1381 [693]
MEDIAN [RANGE]	709 [162-1700]	921 [202-3200]	1210 [250-3750]

Within each of the APACHE II score ranges the distribution of cortisol concentrations remained non-parametric [Table 4.2], although each distribution approximated more closely than the original distribution to normal.

Table 4.2. The distribution of admission plasma cortisol concentrations according to APACHE II scores.

CORTISOL [nmol/L]	APACHE II SCORE GROUPS			TOTAL
	< 16	16 - 24	> 24	
< 500	29	23	9	61
500 - 1000	41	36	15	92
1000 - 1500	16	25	19	60
1500 - 2000	3	14	12	29
2000 - 2500	0	3	6	9
> 2500	0	4	5	9
TOTAL	89	105	66	260

Since, the distributions of the cortisol concentrations were non-parametric, logarithmic transformations were performed in order to define normal ranges. Logarithmic transformation of all admission cortisol measurements [Fig 4.3] and the distributions within each of the APACHE II score ranges resulted in an approximately normal distributions. Normal ranges were calculated using the standard deviations of the log distributions [Table 4.3].

Table 4.3. The overall ranges of plasma cortisol concentrations and the ranges within APACHE II score groups.

CORTISOL RANGE [nmol/L]	APACHE II SCORE GROUP			
	ALL CASES	< 16	16 - 24	>24
95% (2SD)	220 - 3000	200 - 1520	260 - 2080	400 - 3200
99% (3SD)	180 - 3500	160 - 1700	200 - 2800	320 - 4000

Admission plasma cortisol concentration was higher in patients who subsequently died than in those who survived [Table 4.4]. There was a wide range of cortisol concentrations in both groups with considerable overlap of values despite significantly different mean [MD = 673 nmol/L, 95% CI = 540 - 806] and median [P < 0.0001, Wilcoxon's Rank Sum test] concentrations.

Table 4.4. Admission plasma cortisol concentration and APACHE II scores in survivors and nonsurvivors.

	SURVIVED	DIED	TOTAL
NUMBERS	172	88	260
CORTISOL [nmol/L]:			
MEAN [SD]	785 [423]	1458 [658]	1012 [521]
MEDIAN [RANGE]	716 [162-2950]	1260 [180-3750]	916 [162-3750]
MEAN [SD] APACHE SCORE	17.0 [8.0]	26.5 [9.0]	20.2 [9.6]

When plasma cortisol measurements were split into 500 nmol/L ranges [Table 4.5, Fig 4.4], there was a significant difference in mortality among patients in the different admission cortisol groups [χ^2 for linear trend = 31, $P < 0.001$].

Table 4.5. Mortality in ICU patients divided into 6 groups dependent upon admission plasma cortisol concentration.

CORTISOL [nmol/L]	SURVIVED [n]	DIED [n]	MORTALITY % [95%CI]
< 500	52	9	15 [7-26]
500 - 1000	75	17	18 [11-28]
1000 - 1500	34	26	43 [30-57]
1500 - 2000	7	22	76 [57-90]
2000 - 2500	3	6	67 [30-92]
> 2500	1	8	89 [50-99]
TOTAL	172	88	34 [28-40]

Table 4.6 shows that mortality increases over the three APACHE II score groups. If patients are further grouped according to their admission cortisol concentration, as well as their APACHE II score [Figs 4.5 and 4.6], then it can be seen that mortality decreases with decreasing cortisol concentration in the <16 and 16-24 APACHE II score groups (χ^2 for linear trend: 4.2, $P<0.05$ and 19.4, $P<0.001$ respectively). There is no similar trend in patients with APACHE II scores > 24 [Table 4.6]. Patients with cortisol concentra-

tions of less than 500 nmol/L and APACHE II scores of greater than 24 have a high mortality. Although this group is small, the mortality is significantly higher than that of patients with higher cortisol concentrations [500-1000 nmol/L], but the same range of APACHE II scores [80% vs 27%, Fisher exact test (1 tailed) P = 0.01].

Table 4.6. Mortality in patients grouped according to admission plasma cortisol concentrations and APACHE II scores.

		% MORTALITY [DEATHS/CASES]					
APACHE II SCORES:		< 16		16 - 24		>24	
CORTISOL [nmol/L]							
<hr/>							
< 500	4	[1/29]	9	[2/23]	80	[8/10]	
500 - 1000	15	[6/41]	17	[6/36]	27	[4/15]	
1000 - 1500	19	[3/16]	44	[11/25]	67	[12/18]	
> 1500	33	[1/3]	76	[16/21]	83	[19/23]	
All patients	12	[11/89]	33	[35/105]	64	[42/66]	
<hr/>							

Mortality among 7 patients in the lower two APACHE II score groups with admission plasma cortisol concentrations more than two standard deviations below the mean cortisol concentration in their APACHE II score groups was 71% [95% CI, 29-96] compared with 22% [95% CI, 16-28] mortality in the remainder of those groups [Fisher exact test (1 tailed), P < 0.01].

There was no correlation between plasma cortisol concentration and the time of day at which the sample was taken.

Plasma cortisol concentration did not correlate with length of illness prior to ICU admission, age, sex, or arterial pO_2 , but correlated significantly with plasma urea concentration [$r = 0.29$, $P < 0.05$]. Patients with renal failure [urea > 30 mmol/L] on admission [$N = 41$] had a higher mean [SD] plasma cortisol concentration than those [$N = 219$] with plasma urea concentration of less than 30 mmol/l [1493 (712) nmol/L vs 917 (509) nmol/L, $P < 0.05$]. However, the correlation between urea and cortisol disappeared when the cortisol concentration was corrected for severity of illness by dividing it by the non-renal element of the APACHE II score.

Standard ACTH Stimulation Tests

The combined results of the standard ACTH stimulation tests in septic shock cases and non-shocked cases are outlined in Table 4.7. Two cases in which the 30 minute plasma cortisol measurement was not available were not included in the results.

Table 4.7. Mean [SD] plasma cortisol concentration following standard ACTH stimulation tests in 77 ICU cases.

TIME [minutes]	MEAN [SD] CORTISOL CONCENTRATION [nmol/L]		
	0	30	60
SURVIVED [N=42]	731 [347]	1039 [467]	1147 [398]
DIED [N=35]	893 [477]	1102 [444]	1114 [423]
TOTAL [N=77]	803 [418]	1067 [458]	1132 [410]

In survivors and nonsurvivors, both the 30 and 60 minute plasma cortisol levels were significantly higher than the basal levels. The range of cortisol responses to ACTH varied from -80 nmol/L to 1000 nmol/L. The mean cortisol response was 358 nmol/L [SD = 205]. The basal cortisol concentration in nonsurvivors was higher than survivors [MD = 162 nmol/L, 90% CI = 8 - 316] but this was not significant at the 95% level of confidence. The cortisol response in survivors was significantly higher than in nonsurvivors [449 nmol/L vs 248 nmol/L, MD = 201 nmol/L, 99% CI = 69 - 333], but there was no difference between the stimulated cortisol concentrations.

The basal plasma cortisol concentration did not correlate with APACHE II scores, but there was a significant inverse correlation between APACHE II scores and the cortisol response to ACTH ($r = -0.53$, $P < 0.01$). The results of the ACTH stimulation tests stratified by the three APACHE II score groups used above are given in Table 4.8.

Table 4.8. The mean [SD] basal plasma cortisol concentration and cortisol responses to a standard ACTH stimulation test stratified by APACHE II scores.

APACHE II SCORES:	<16	16 - 24	>24
Number of cases	17	32	28
Basal cortisol (nmol/L)	793 [327]	844 [346]	766 [382]
Cortisol response (nmol/L)	456 [269]	381 [211]	272 [183]

The basal plasma cortisol concentration did not vary signif-

icantly across the three APACHE II score groups. The mean plasma cortisol response to ACTH was lower in patients with an APACHE II score >24 than in the other two groups [<16 vs >24 : MD = 184 nmol/L, 95% CI = 48 - 320. $16-24$ vs >24 : MD = 109 nmol/L, 95% CI = 7 - 212].

Overall, there was a significant correlation between basal plasma cortisol concentration and the cortisol increment [$r = -0.271$, $P<0.05$]. In nonsurvivors, the correlation was more marked [$r = -0.41$, $P<0.02$], but there was no significant correlation in survivors. There was no significant correlation between either basal cortisol concentration or cortisol increment and age, and no difference between the sexes.

Table 4.9. Mortality among patients stratified by APACHE II score and the cortisol response to a standard ACTH stimulation test.

APACHE II SCORES:	<16	16 - 24	>24	Total
Cortisol increment < 250 nmol/l:				
Number of cases	4	8	13	25
Deaths (n)	2	6	11	19
Mortality (%)	50	75	85	76
Cortisol increment > 250 nmol/l:				
Number of cases	13	24	15	52
Deaths (n)	2	7	7	16
Mortality (%)	15	29	47	31
Totals:				
Number of cases	17	32	28	77
Deaths (n)	4	13	18	35
Mortality (%)	23	41	64	45

Mortality was significantly higher in patients with a cortisol increment of less than 250 nmol/l [76%, 95% CI 55-91] than in patients with a greater increment [35%, 95% CI 19-45]. The same trend was present in each of the three APACHE II score groups [Table 4.9] and was statistically significant in the 16-24 and > 24 APACHE II score groups (Fisher exact test, 1-tailed, both $P < 0.05$).

The results in the non-shocked cases and the septic shock cases were also analysed separately.

Non-Shocked Cases

Forty seven non-shocked patients were investigated and data were complete in 45 cases [Figs 4.7 - 4.9]. The Mean (SD) APACHE II score of patients studied was 18.2 (6.6). In 3 of the 45 cases investigated the plasma cortisol concentration did not increase during the test. In a further 5 cases the cortisol increase was less than 200 nmol/L. In 12 cases the cortisol increase was greater than 500 nmol/L. Overall, the mean plasma cortisol concentration was significantly higher than the mean basal concentration at 30 minutes [MD = 293 nmol/L, 99% CI = 86 - 491], and 60 minutes [MD = 343 nmol/L, 99% CI = 134 - 522, Table 4.10]. There was no significant difference between the mean cortisol concentrations at 30 and 60 minutes.

In survivors [Fig 4.7] and non-survivors [Figs 4.8 and 4.9], the 30 and 60 minute cortisol levels were significantly higher than basal [Nonsurvivors - 30 mins: MD = 244

nmol/L, 95% CI = 106 - 383, 60 mins: MD = 267 nmol/L, 95% CI = 117 - 432. Survivors - 30 mins: MD = 312 nmol/L, 99% CI = 147 - 466. 60 mins: MD = 381 nmol/L, 99% CI = 164 - 598]. The mean [SD] cortisol response was 371 [218] nmol/L. The response was significantly greater in survivors than nonsurvivors [mean (SD) = 413 (212) nmol/L vs 289 (223) nmol/L, MD = 123 nmol/L, 95% CI = 2 - 257]. Basal cortisol concentration was significantly higher in non-survivors than survivors [mean (SD) = 1028 (488) nmol/L vs 746 (335) nmol/L, MD = 282 nmol/L, 95% CI = 39 - 525].

Table 4.10. Mean [SD] plasma cortisol concentration during a standard ACTH stimulation test in 45 ICU patients.

TIME [minutes]	MEAN [SD] CORTISOL CONCENTRATION [nmol/L]		
	0	30	60
SURVIVED [N=29]	747 [335]	1059 [355]	1128 [338]
DIED [N=16]	1028 [488]	1272 [438]	1295 [414]
TOTAL [N=45]	842 [416]	1135 [400]	1185 [374]

Patients with high basal cortisol concentrations tended to have a smaller cortisol increments than patients with lower basal levels. The correlation between basal plasma cortisol concentration and the cortisol increment was significant [$r = -0.36$, $P < 0.05$]. This negative correlation between basal plasma cortisol concentration and cortisol increment was more marked in survivors [$r = -0.483$, $P < 0.05$, Fig 4.10], and did not reach statistical significance in nonsurvivors

[r = -0.248, P=0.08, Fig 4.10]. There was no significant correlation between basal plasma cortisol concentration and APACHE II scores, but there was a significant negative correlation between APACHE II scores and the cortisol response to ACTH [r = - 5.8, P<0.01].

Septic Shock Cases

Data collection was complete in all 32 cases. The mean (SD) APACHE II score of patients studied was 25.6 (8.4). In 2 cases the plasma cortisol concentration did not increase during the test. In a further 11 cases, the increase was under 250 nmol/L, and in 9 cases the increase was greater than 500 nmol/L [Figs 4.11 and 4.12]. The 30 and 60 minute plasma cortisol levels were both significantly higher than the basal levels [30 min: MD = 291 nmol/L, 95% CI = 71 - 511. 60 min: MD = 309 nmol/L, 99% CI = 23 - 595, Table 4.11].

Table 4.11. Mean [SD] plasma cortisol concentration during a standard ACTH stimulation test in 32 cases of septic shock.

TIME [minutes]	MEAN [SD] CORTISOL CONCENTRATION [nmol/L]		
	0	30	60
SURVIVED [N=13]	692 [372]	1154 [528]	1190 [510]
DIED [N=19]	778 [437]	950 [390]	961 [367]
TOTAL [N=32]	745 [414]	1036 [464]	1054 [445]

Patients who subsequently died had higher basal plasma

cortisol levels than patients who survived, but this did not reach statistical significance. There was no significant difference between the cortisol concentrations in survivors and nonsurvivors at 30 or 60 minutes. In both survivors [Fig 4.11] and nonsurvivors [Fig 4.12], the mean 30 and 60 minute cortisol levels were significantly higher than the mean basal level [Survivors: 30 min, MD = 462 nmol/L, 99% CI = 41 - 832; 60 min, MD = 498 nmol/L, 99% CI = 83 - 859. Nonsurvivors: 30 min, MD = 170 nmol/L, 95% CI = 61 - 310; 60 min, MD = 182 nmol/L, 99% CI = 40 - 323].

The mean [SD] plasma cortisol increment was significantly higher in survivors than nonsurvivors [528 (251) nmol/L vs 213 (200) nmol/L, MD = 315 nmol/L, 99% CI = 96 - 535]. All 13 patients with plasma cortisol increments of less than 250 nmol/L subsequently died. There were 6 deaths among the 19 patients with cortisol responses greater than 250 nmol/L [$P < 0.001$, χ^2 with Yate's correction].

Overall, there was no correlation between the cortisol response and the basal plasma cortisol concentration. However, there was a significant positive correlation between basal cortisol concentration and the cortisol increment in survivors [$r = 0.522$, $P < 0.05$, Fig 4.13], and a significant negative correlation between the cortisol response and the basal cortisol concentration in nonsurvivors [$r = -0.507$, $P < 0.05$, Fig 4.13].

The cortisol response was lower in patients with Gram-negative organisms on blood culture [mean (SD) = 281 (196) nmol/L] than in those with gram-positive organisms or nega-

tive blood cultures [mean (SD) = 407 (236) nmol/L], but this did not reach statistical significance.

Low Dose ACTH Stimulation Tests

Data were complete in the 25 tests performed. The mean (SD) APACHE II scores of the non-shocked cases [Group 2] and the septic shock cases [Group 3] were 16.9 (5.3) and 23.4 (7.1) respectively. Plasma cortisol concentration increased in all control cases [Fig 4.14] and all non-shocked cases following low dose ACTH [Figs 4.15]. Cortisol concentration increased in 7 of the 10 septic shock cases [Fig 4.16].

Table 4.12. Mean [SD] plasma cortisol concentration following a low dose ACTH stimulation test.

TIME [minutes]	MEAN [SD] CORTISOL CONCENTRATION [nmol/L]			
	0	15	30	60
CONTROLS [N=5]	522 [87]	674 [133]	724 [144]	655 [129]
GROUP 2 [N=10]	844 [253]	972 [250]	1008[252]	959 [255]
GROUP 3 [N=10]	702 [309]	754 [284]	765 [277]	744 [297]

Basal cortisol concentration was significantly higher in the ICU patients than in the controls [Group 2: MD = 322 nmol/L, 95% CI = 60 - 578. Group 3: MD = 306 nmol/L, 95% CI = 119 - 493, Table 4.12]. In the control cases, plasma cortisol concentration was significantly higher the than basal con-

centration, at 15, 30 and 60 minutes after injection of the ACTH [15 mins: MD = 152 nmol/l, 99% CI = 17-287. 30 mins: MD = 182 nmol/l, 99% CI = 83-311. 60 mins: MD = 133 nmol/l, 99% CI = 28-239]. In the non-shocked patients, plasma cortisol concentration was higher than basal at 15, 30 and 60 minutes [15 mins: MD = 128 nmol/L, 99% CI = 89-168. 30 mins: MD = 162 nmol/L, 95% CI = 112-212. 60 mins: MD = 114 nmol/L, 95% CI = 62-165]. In the septic shock cases, the increase in mean cortisol concentration was smaller than in the non-shocked cases, but was still significant [15 mins: MD = 62 nmol/L, 95% CI = 22-101. 30 mins: MD = 91 nmol/L, 95% CI = 27-154. 60 mins: MD = 66 nmol/L, 95% CI = 5-127].

The mean [SD] cortisol increments in the 3 groups were: controls, 204 [38] nmol/L; non-shocked cases, 168 [46] nmol/L; septic shock cases, 76 [50] nmol/L. There was no significant difference between the responses in the controls and in the non-shocked cases, but the mean response in the septic shock cases was significantly lower than in the controls [MD = 128 nmol/l, 99% CI = 51-205] and the non-shocked cases [MD = 92 nmol/l, 99% CI = 30-154]. There was no difference in the cortisol increment between survivors and nonsurvivors in either group of cases.

Depot ACTH Stimulation Tests

Data collection was complete in the 5 cases and 5 controls investigated. The mean (SD) APACHE II score of the septic shock cases was 23.5 (6.2). In both the control group and the septic shock group, plasma cortisol concentration in-

creased in all cases between basal and 1 hour and between 1 hour and 2 hours [Figs 4.17]. The basal plasma cortisol concentrations were similar in both groups [Table 4.13]. Two hours after ACTH administration plasma cortisol concentrations were significantly higher in the controls than in the septic shock patients [MD = 156 nmol/L, 95% CI = 16 - 314]. Mean plasma cortisol concentration remained significantly higher in the control group for the remainder of the test, the difference between the 2 groups steadily increasing [3 hours, MD = 268 nmol/L, 95% CI = 74 - 462; 4 hours, MD = 462 nmol/L, 95% CI = 104 - 820; 5 hours, MD = 566 nmol/L, 95% CI = 166 - 966].

Table 4.13. Mean [SD] plasma cortisol concentration during depot ACTH stimulation tests in septic shock cases and healthy controls.

TIME [h]	MEAN [SD] CORTISOL CONCENTRATION [nmol/L]					
	0	1	2	3	4	5
CASES	452[111]	642[141]	817[124]	861[175]	816[307]	788[306]
CONTROLS	458[61]	808[93]	982[75]	1129[68]	1278[162]	1354[239]

In the control group, plasma cortisol concentration increased between 2 and 3 hours in all cases, but fell in 2 of the septic shock cases. Mean plasma cortisol concentration was significantly higher at 3 hours than at 2 hours in the control group [MD = 147 nmol/L, 99% CI = 116 - 177], but not in the septic shock group. In the septic shock group plasma

cortisol concentration fell between 3 and 5 hours in 4 cases compared with only 1 case in the control group.

The mean plasma cortisol concentration in controls remained within the expected range for the duration of the test. In the septic shock group the mean cortisol concentration fell below the lower limit of normal after 3 hours [Fig 4.18].

Serial Plasma Cortisol Measurement

Data collection was incomplete in 2 cases. The mean (SD) APACHE II score of patients studied was 23.6 (4.9). Of the 130 samples taken for cortisol assay, measurements were available in 127. Case 4 received hydrocortisone replacement when, after a downward trend for 48 hours, the plasma cortisol concentration fell below 200 nmol/l [Fig 4.19]. This case will be excluded from the analysis. Of the remaining 9 cases, 4 survived and 5 died. The mean period from initial investigation of survivors to ICU discharge was 9.5 days. Excluding case 4, the mean period from initial investigation of nonsurvivors to death was 3.5 days.

There was no significant difference in plasma cortisol concentration between initial measurement and measurement before death or discharge. There was no consistent pattern in cortisol measurement in either survivors or nonsurvivors. In 2 nonsurvivors [cases 4 and 20] plasma cortisol concentration fell below 200 nmol/l on at least one occasion.

Repeat Standard ACTH Stimulation Tests on Recovery

Data were complete in the 10 cases studied. It was not pos-

sible to calculate APACHE II scores at the time of the repeat test because of insufficient data. All patients had a basal cortisol concentration greater than 250 nmol/L, and no patient had a cortisol increment following ACTH of less than 250 nmol/L [Table 4.14].

Table 4.14. Mean [SD] plasma cortisol concentration during a standard ACTH stimulation test on recovery.

MEAN [SD] PLASMA CORTISOL CONCENTRATION [nmol/L]			
TIME [minutes]	0	30	60
ORIGINAL TEST	629 [341]	911 [418]	1023 [381]
RECOVERY TEST	435 [246]	787 [219]	933 [326]

The basal cortisol concentrations were not significantly different. The cortisol increment during the recovery test [mean (SD) = 509 (186) nmol/L] was greater than during the original test [mean (SD) = 421 (89) nmol/L], but this did not quite reach statistical significance [MD = 88, 95% CI = -15 - 177].

FIG 4.1. THE DISTRIBUTION OF ADMISSION PLASMA CORTISOL CONCENTRATION IN 260 CASES.

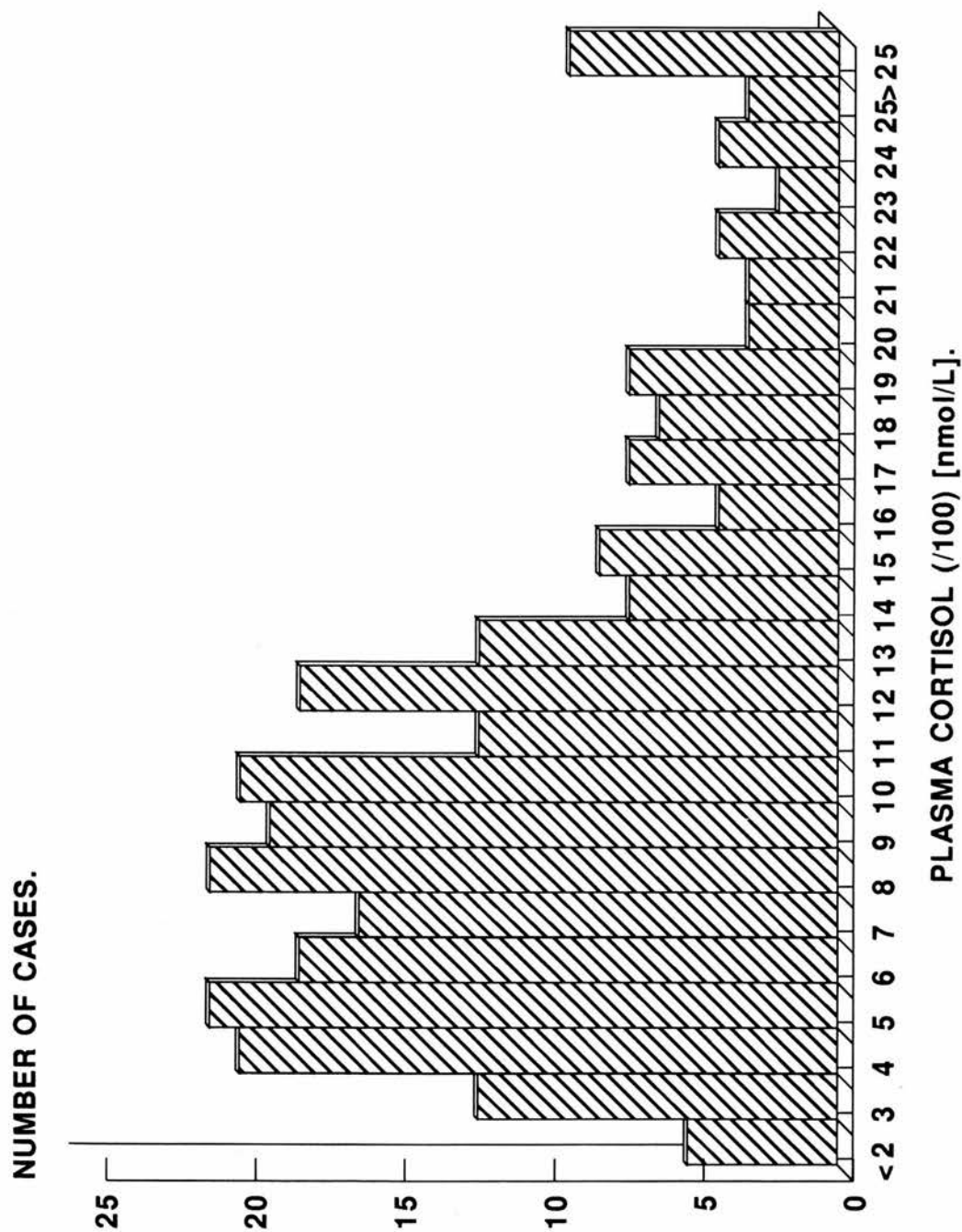


FIG 4.2. THE DISTRIBUTION OF ADMISSION PLASMA CORTISOL
CONCENTRATION IN 260 CASES.
LOGARITHMIC SCALE

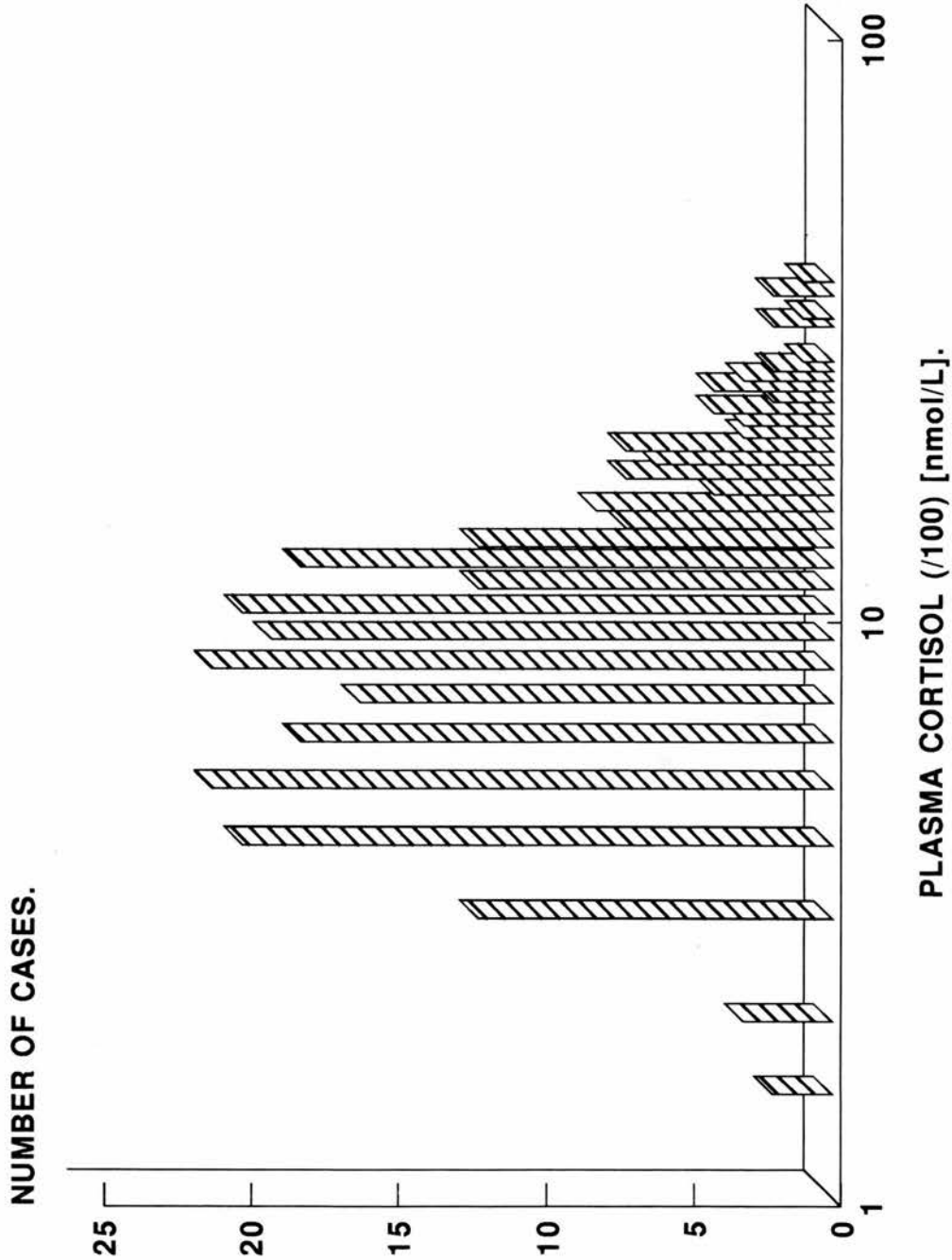
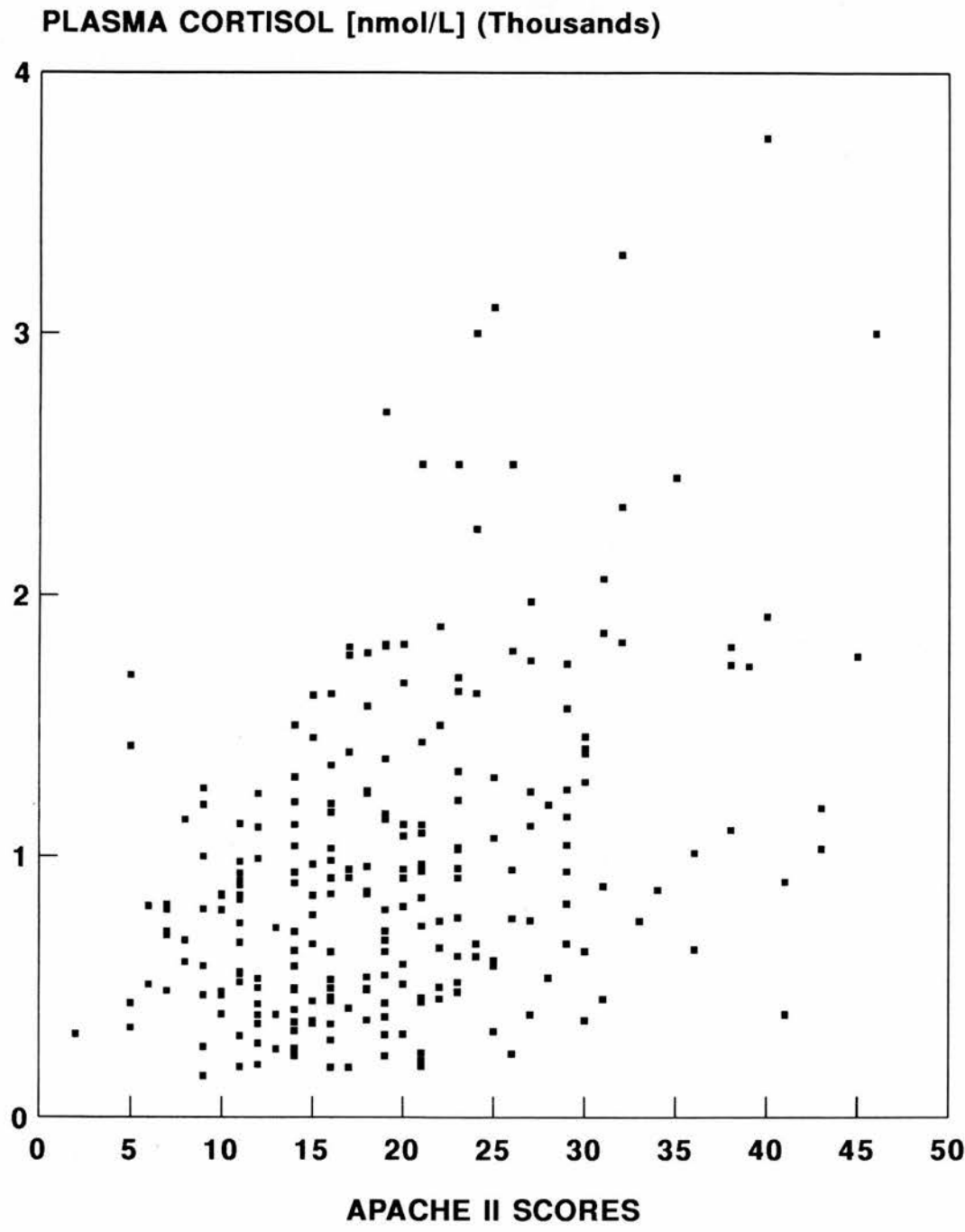


FIG 4.3. ADMISSION PLASMA CORTISOL CONCENTRATION VERSUS APACHE II SCORE.



**FIG 4.4. MORTALITY IN PATIENTS GROUPED
ACCORDING TO ADMISSION PLASMA CORTISOL
CONCENTRATION**

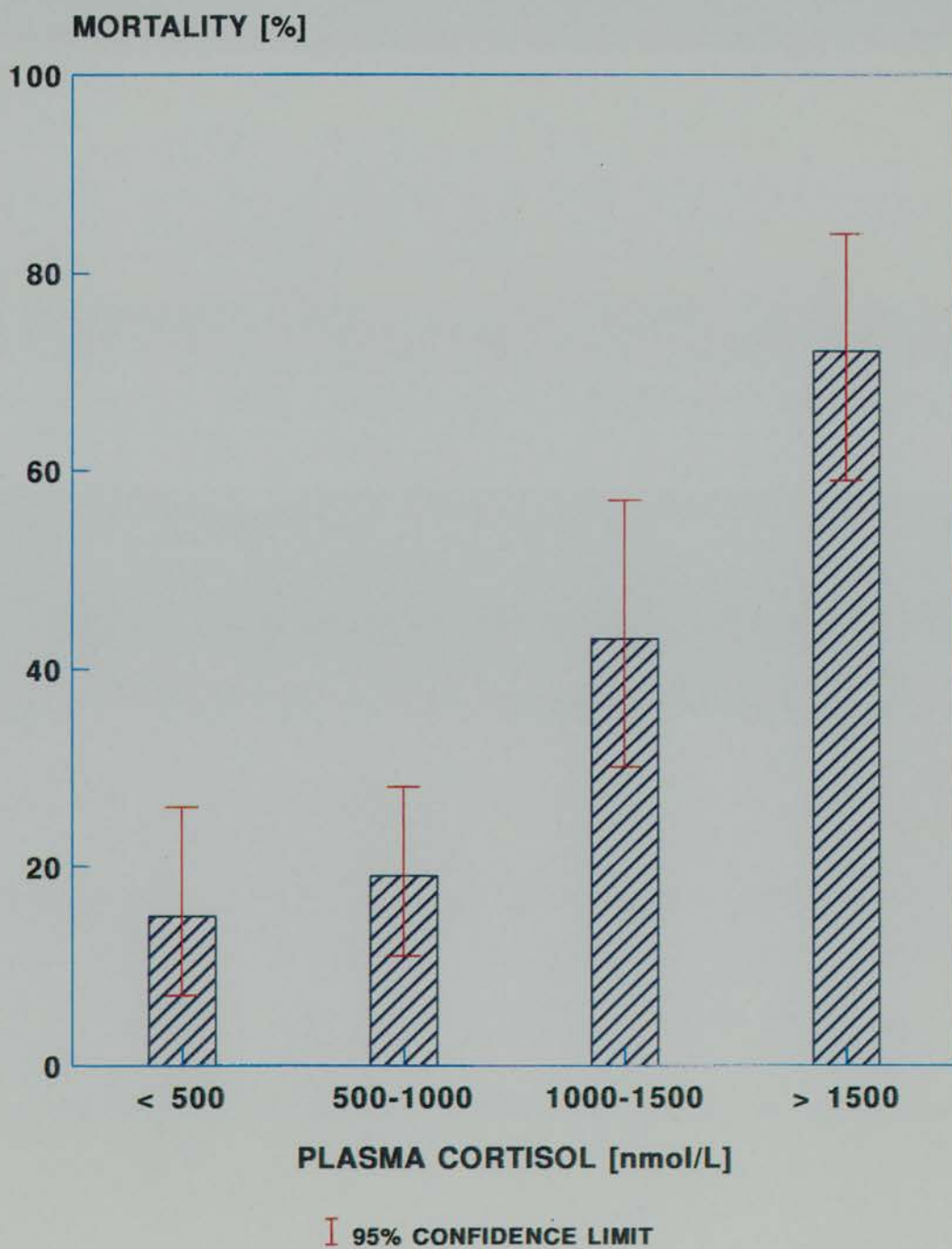


FIG 4.5. THE DISTRIBUTION OF APACHE II SCORES IN PATIENTS GROUPED ACCORDING TO ADMISSION CORTISOL CONCENTRATION.

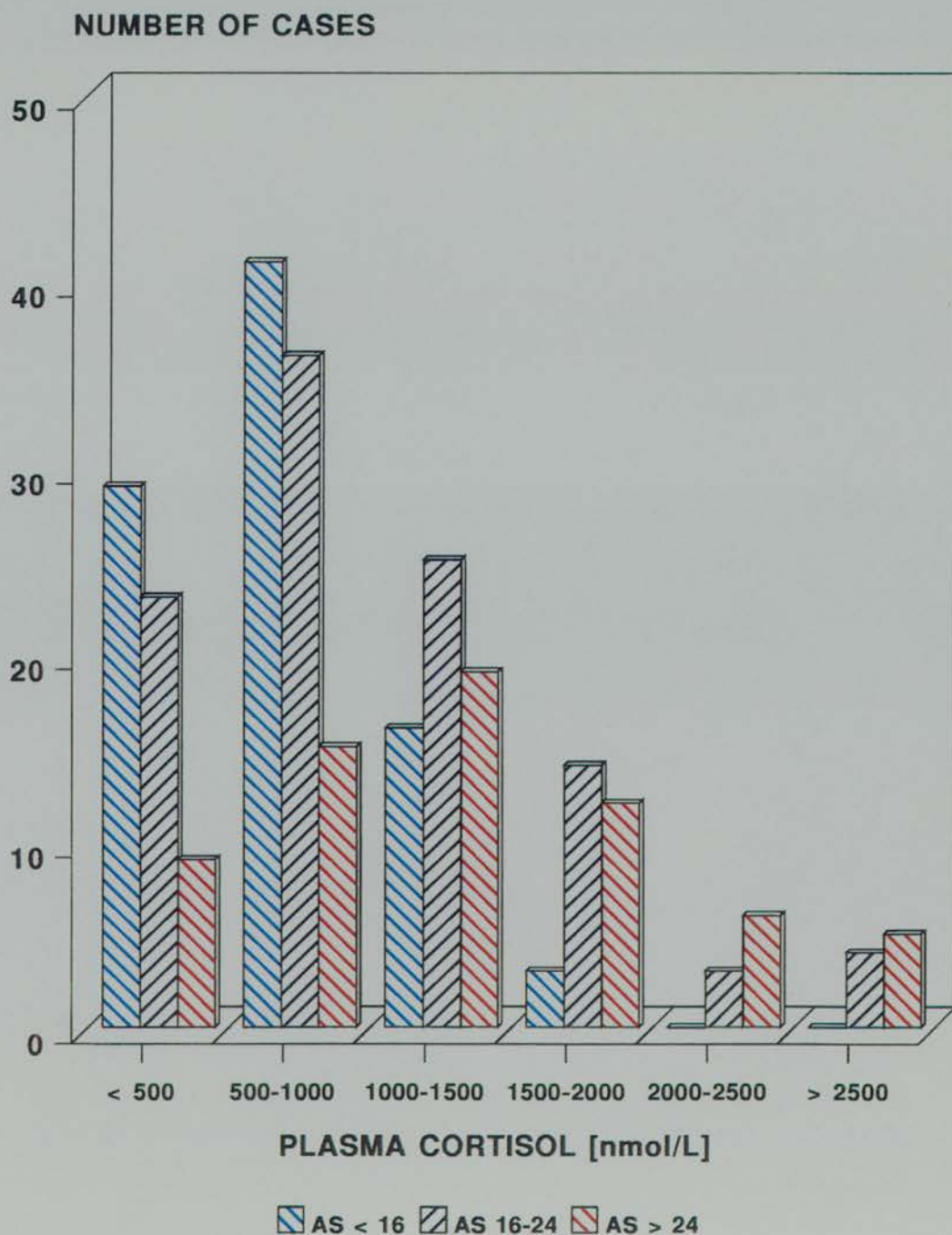


FIG 4.6. MORTALITY IN PATIENTS GROUPED ACCORDING TO ADMISSION CORTISOL CONCENTRATION AND APACHE II SCORE.

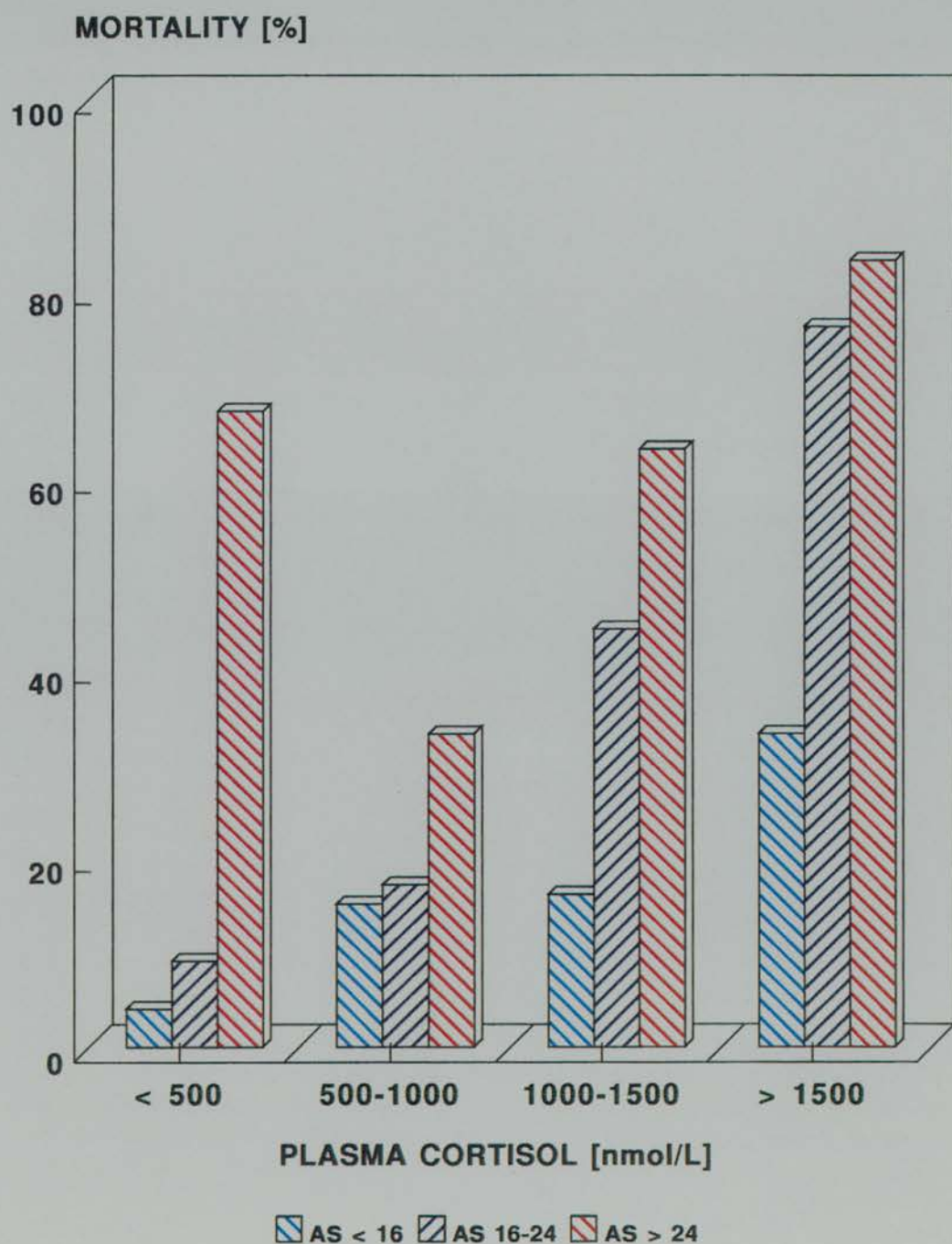


FIG 4.7. STANDARD ACTH STIMULATION TESTS
IN SURVIVORS

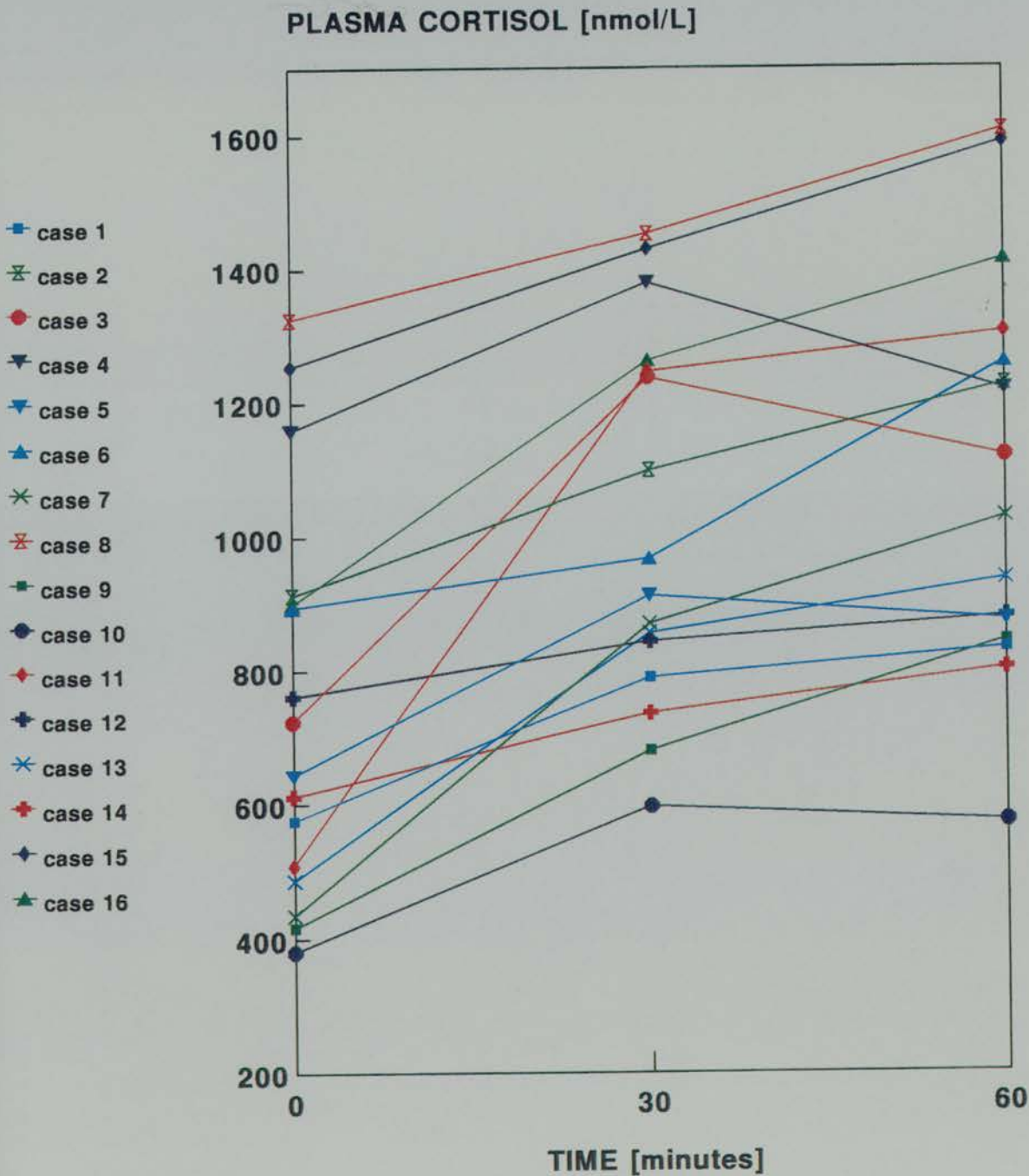
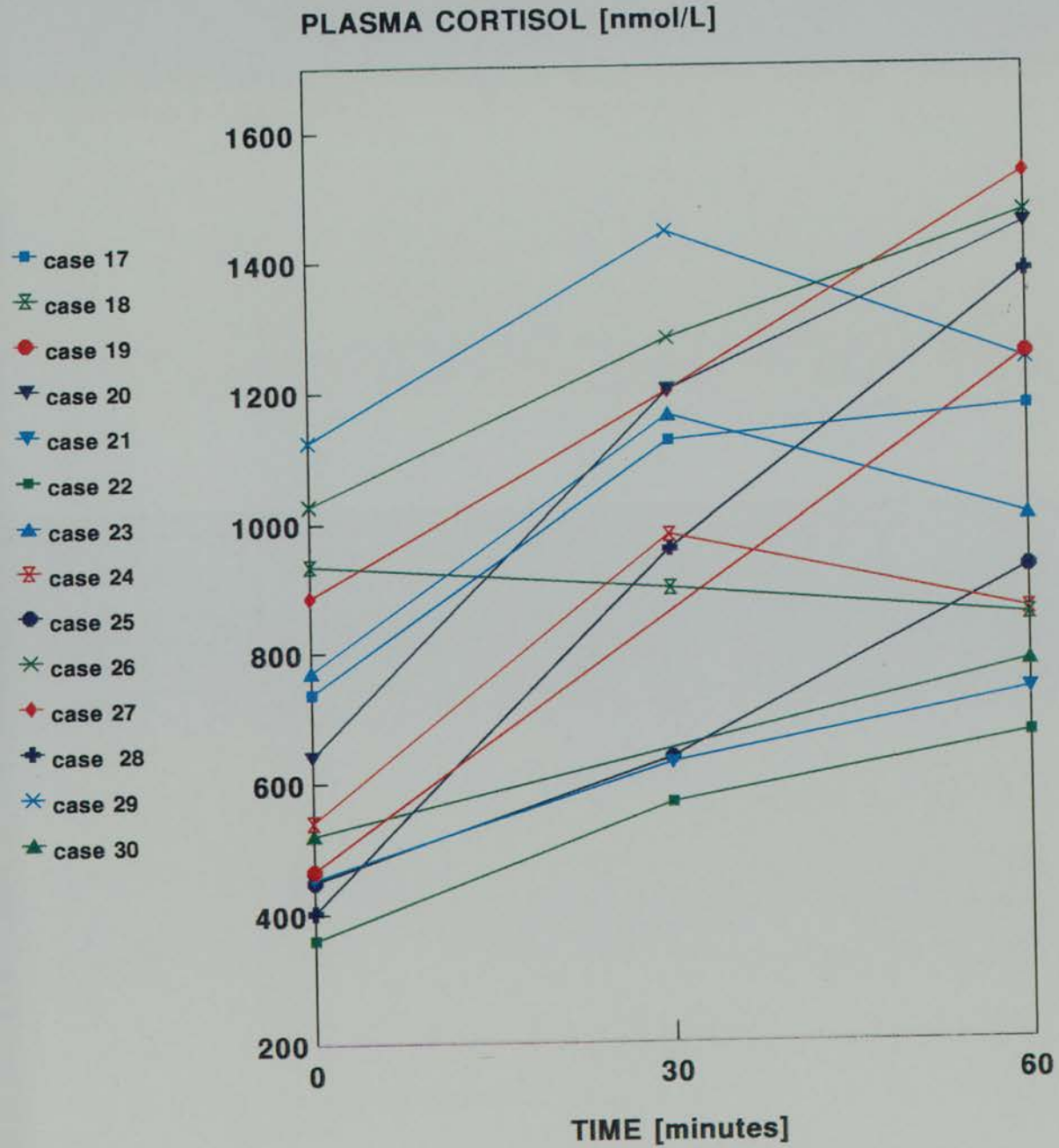


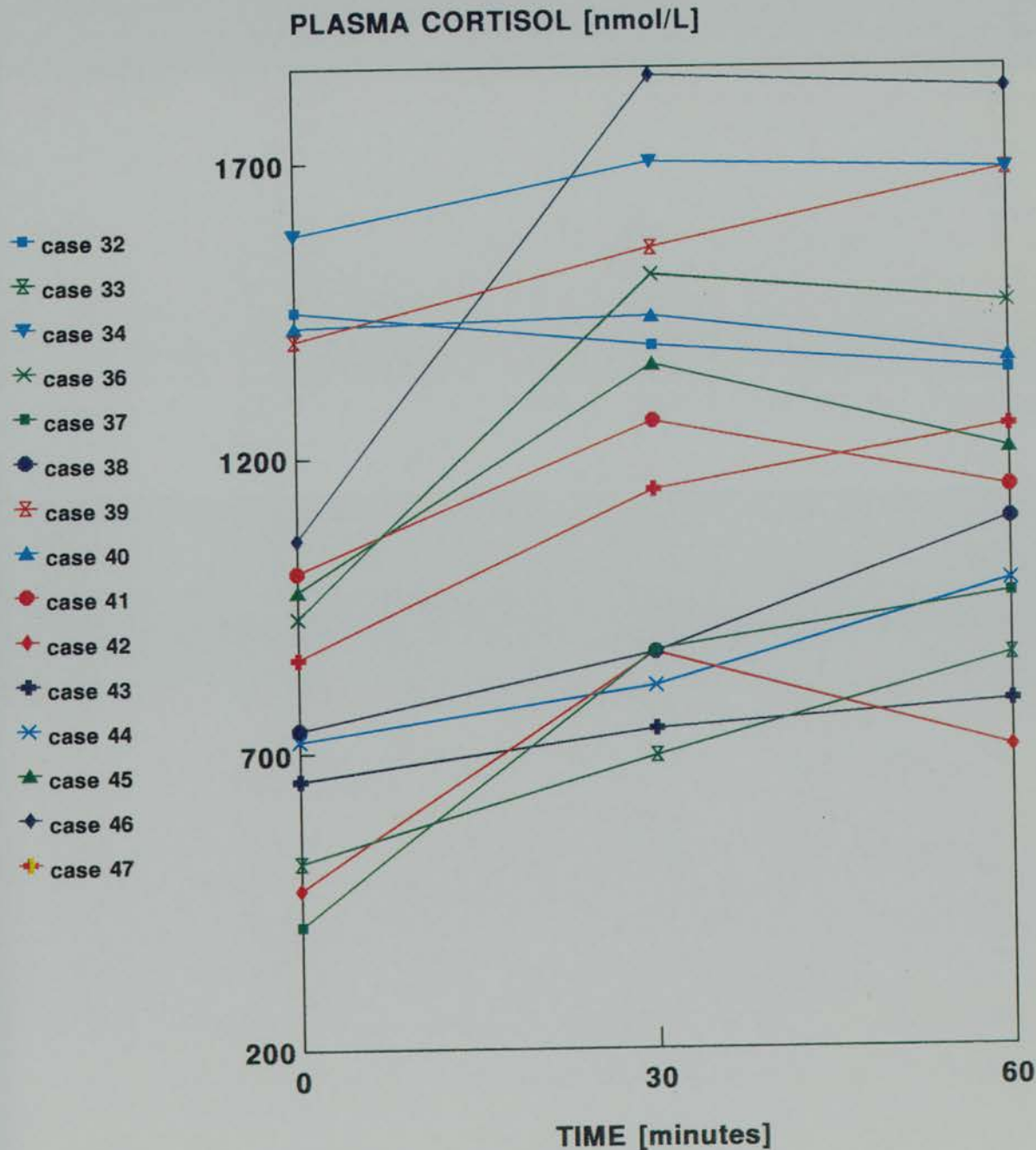
FIG 4.8. STANDARD ACTH STIMULATION TESTS
IN SURVIVORS



MISSING DATA IN CASES 19 AND 30.

CASE 31 [NOT SHOWN]: 0 MINS, 1850 nmol/L; 30 MINS, 2310 nmol/L; 60 MINS, 2143 nmol/L.

FIG 4.9 STANDARD ACTH STIMULATION TESTS
IN NONSURVIVORS



CASE 35 [NOT SHOWN]: 0 MINS, 2340 nmol/L; 30 MINS, 2294 nmol/L; 60 MINS, 2320 nmol/L.

FIG 4.10. PLASMA CORTISOL INCREMENT FOLLOWING ACTH STIMULATION VERSUS BASAL CORTISOL CONCENTRATION

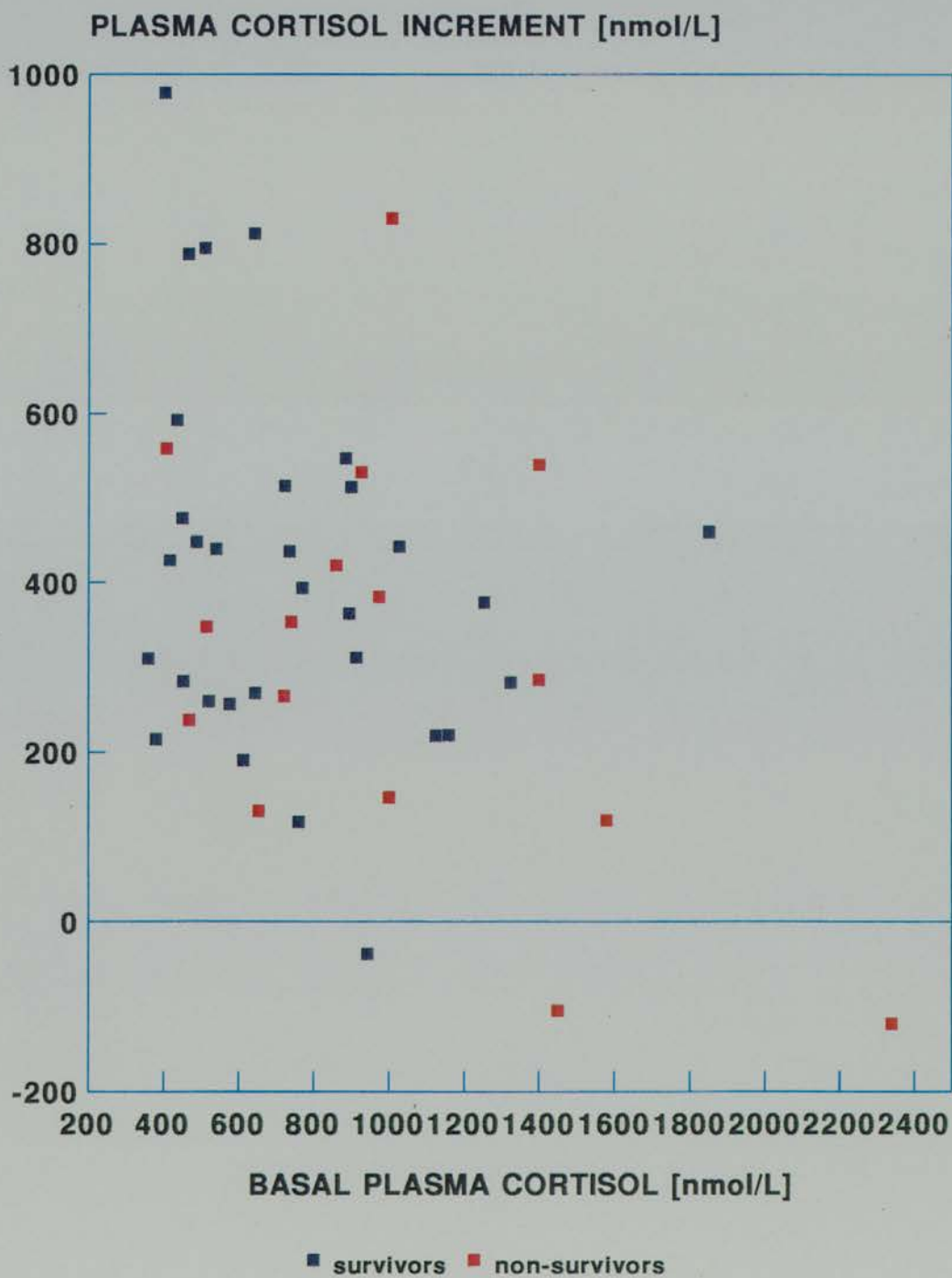


FIG 4.11. STANDARD ACTH STIMULATION TESTS IN SEPTIC SHOCK SURVIVORS

PLASMA CORTISOL [nmol/L]

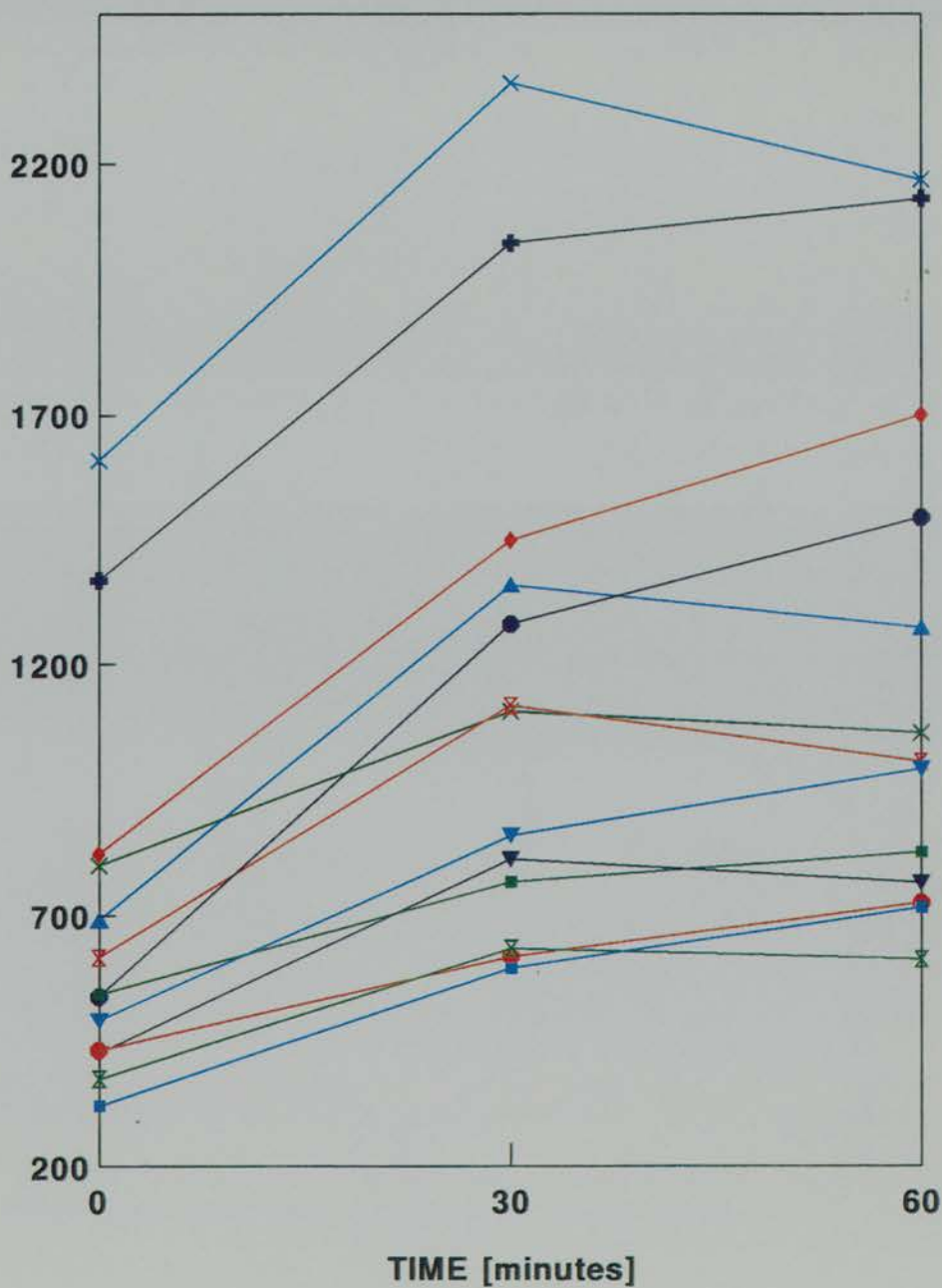
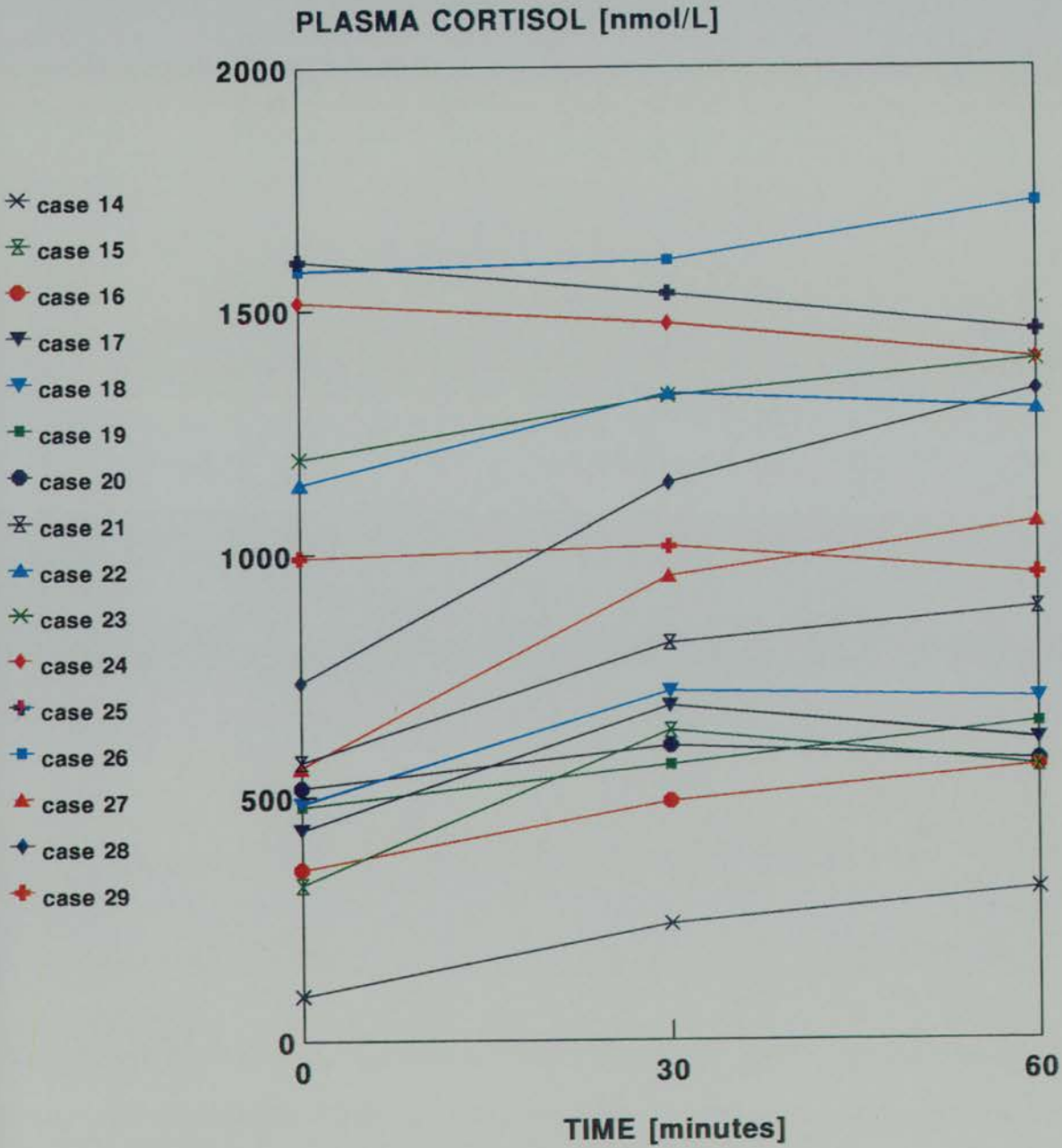


FIG 4.12. STANDARD ACTH STIMULATION TESTS IN SEPTIC SHOCK NONSURVIVORS



CASE 30 [NOT SHOWN]: 0 MINS, 596 nmol/L; 60 MINS, 1208 nmol/L.
CASE 31 [NOT SHOWN]: 0 MINS, 663 nmol/L; 30 MINS, 727 nmol/L; 60 MINS, 689 nmol/L.
CASE 32 [NOT SHOWN]: 0 MINS, 994 nmol/L; 30 MINS, 1263 nmol/L; 60 MINS, 957 nmol/L.

FIG 4.13. PLASMA CORTISOL INCREMENT FOLLOWING ACTH STIMULATION VERSUS BASAL CORTISOL CONCENTRATION IN SEPTIC SHOCK

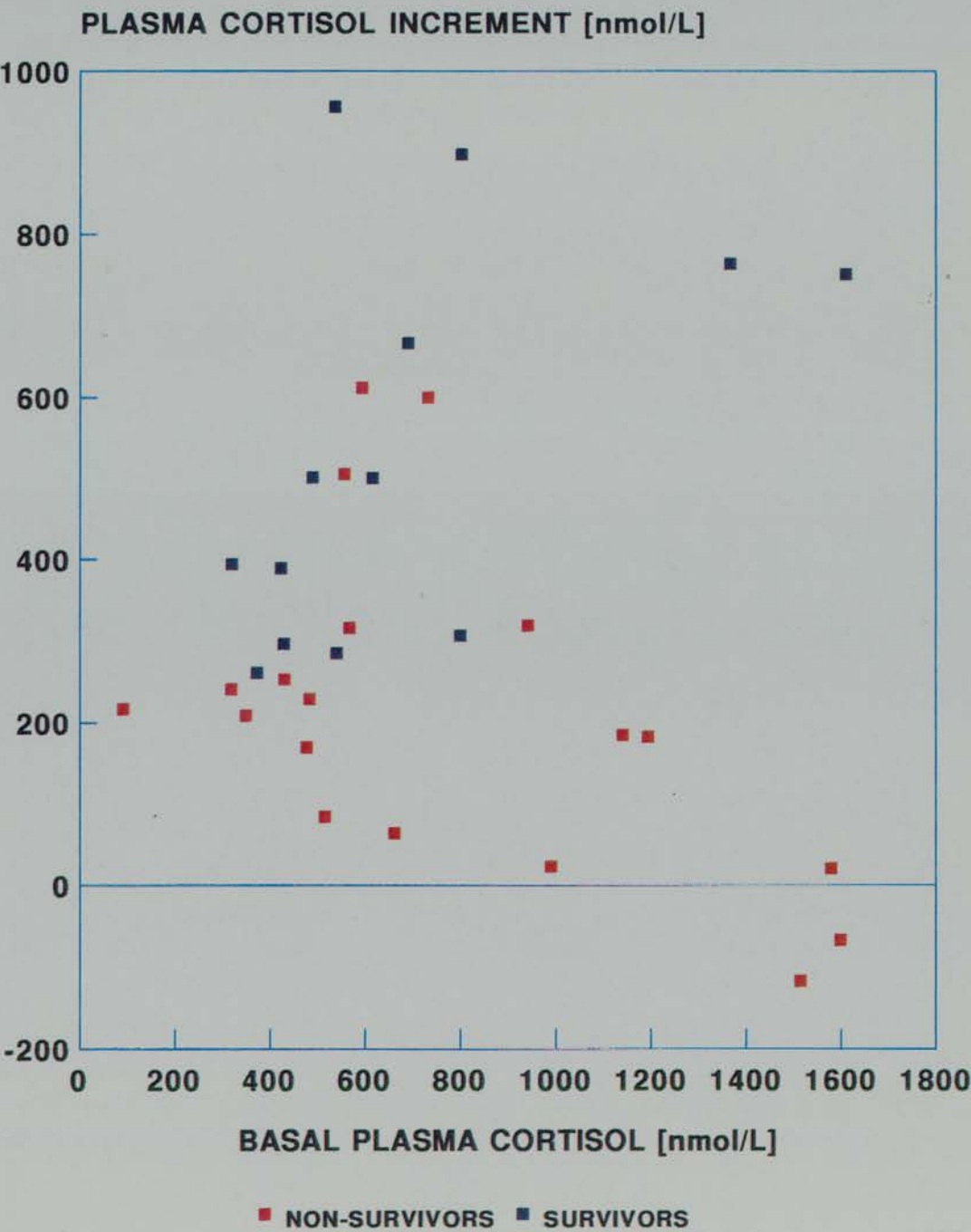


FIG 4.14. LOW DOSE ACTH STIMULATION TESTS
IN CONTROLS

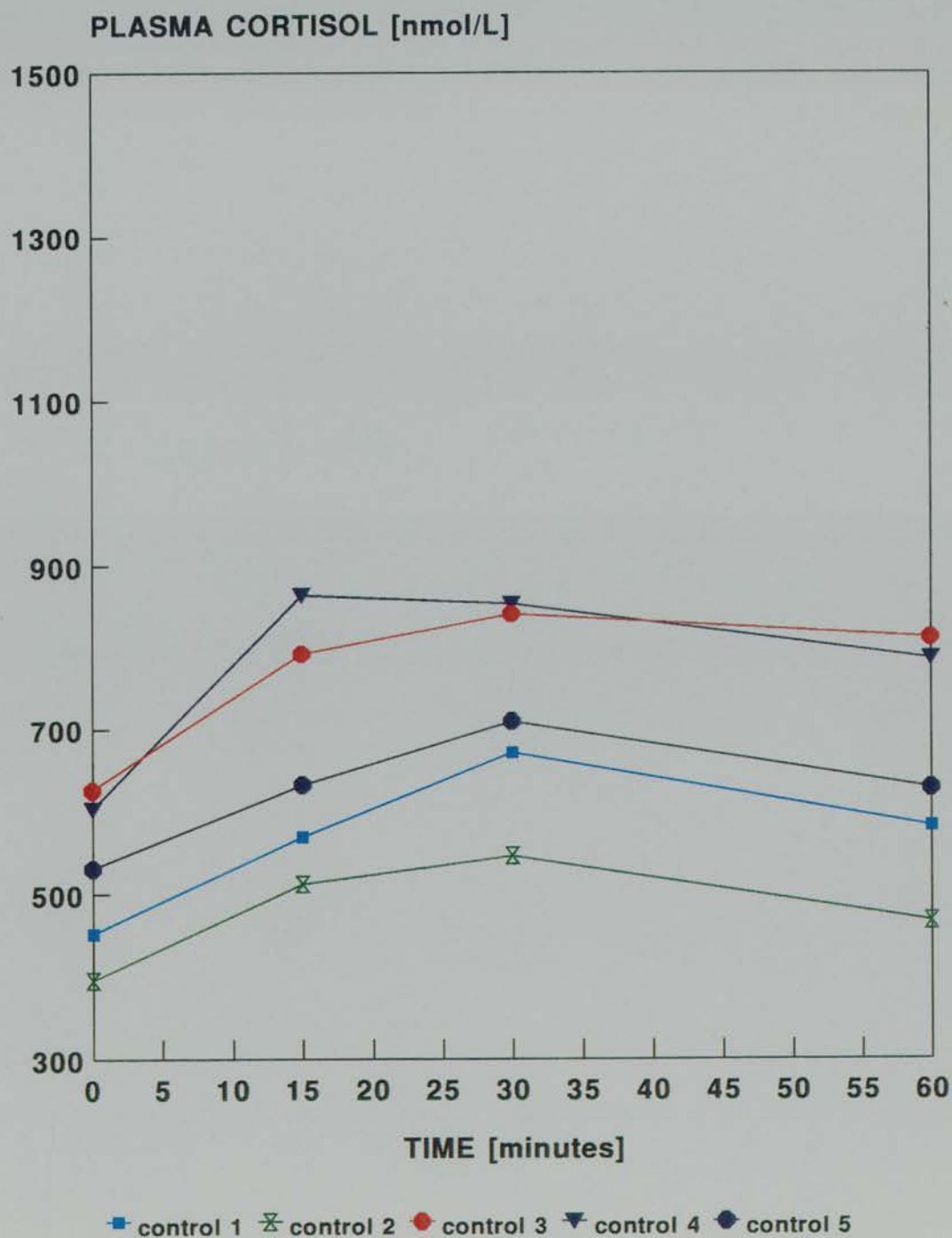


FIG 4.15. LOW DOSE ACTH STIMULATION TESTS
IN GROUP 2

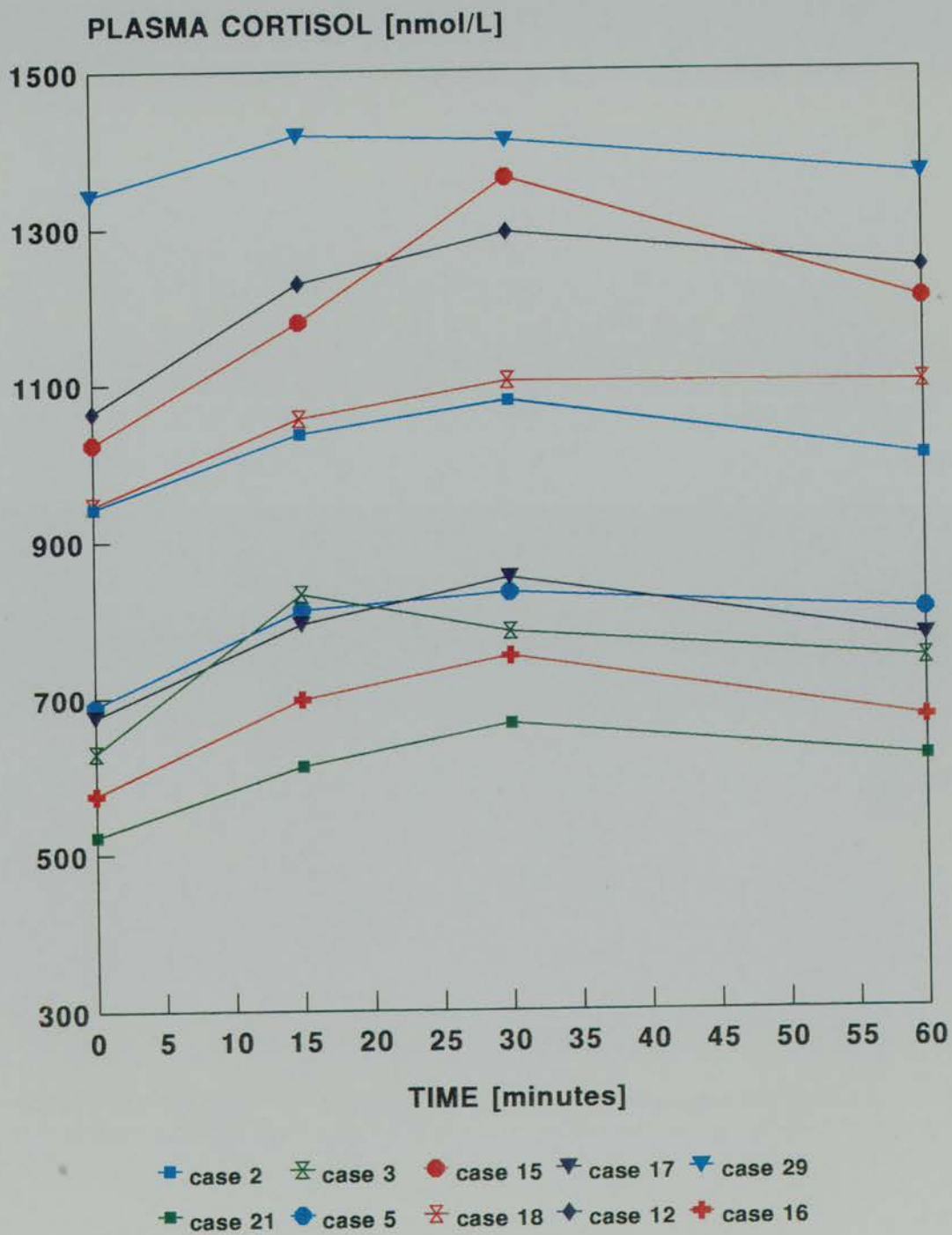
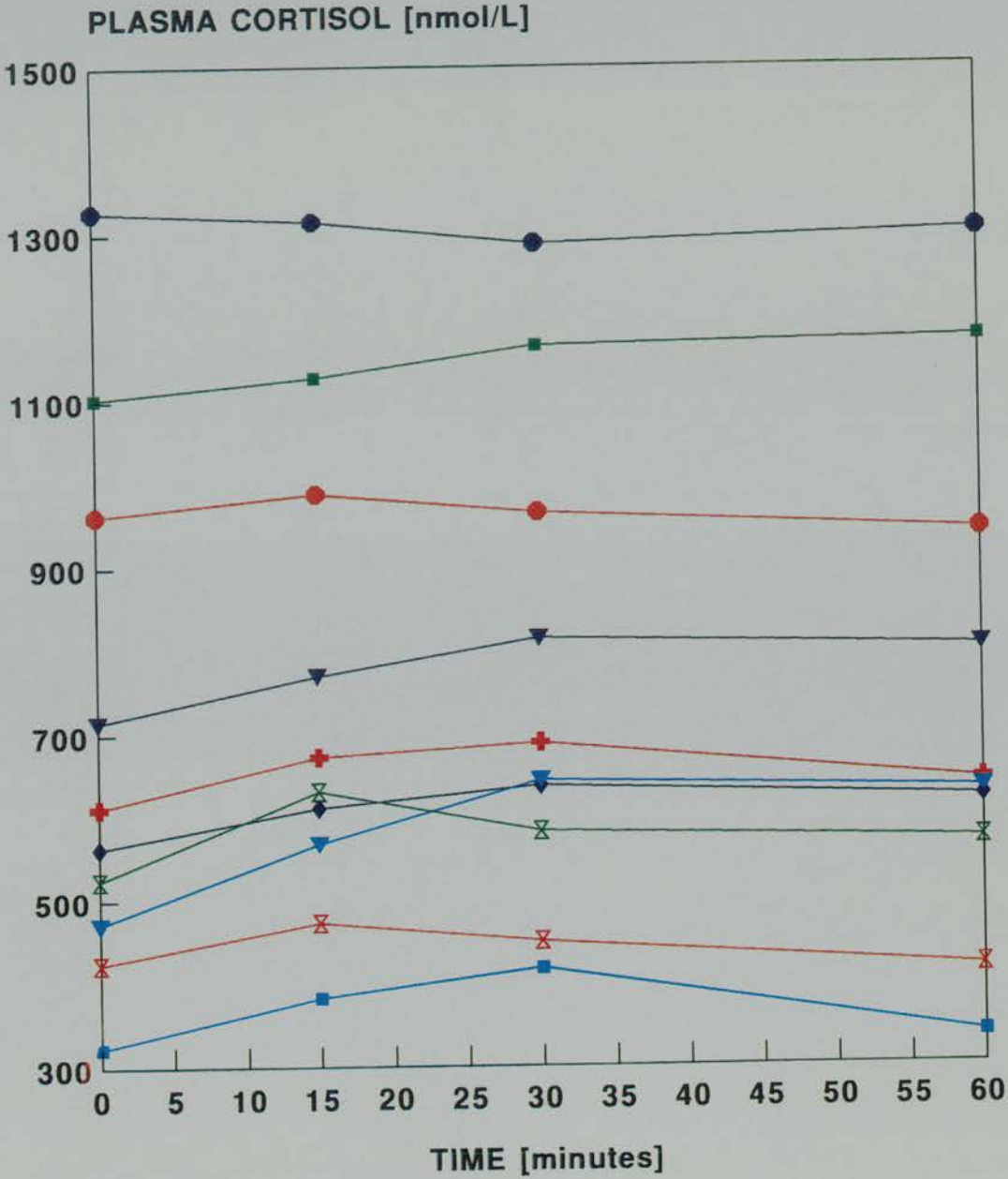


FIG 4.16. LOW DOSE ACTH STIMULATION TEST3
IN GROUP 3



case 2 case 5 case 8 case 10 case 13
 case 16 case 19 case 2 case 9 case 6

FIG 4.17. DEPOT ACTH STIMULATION TESTS
IN CASES AND CONTROLS

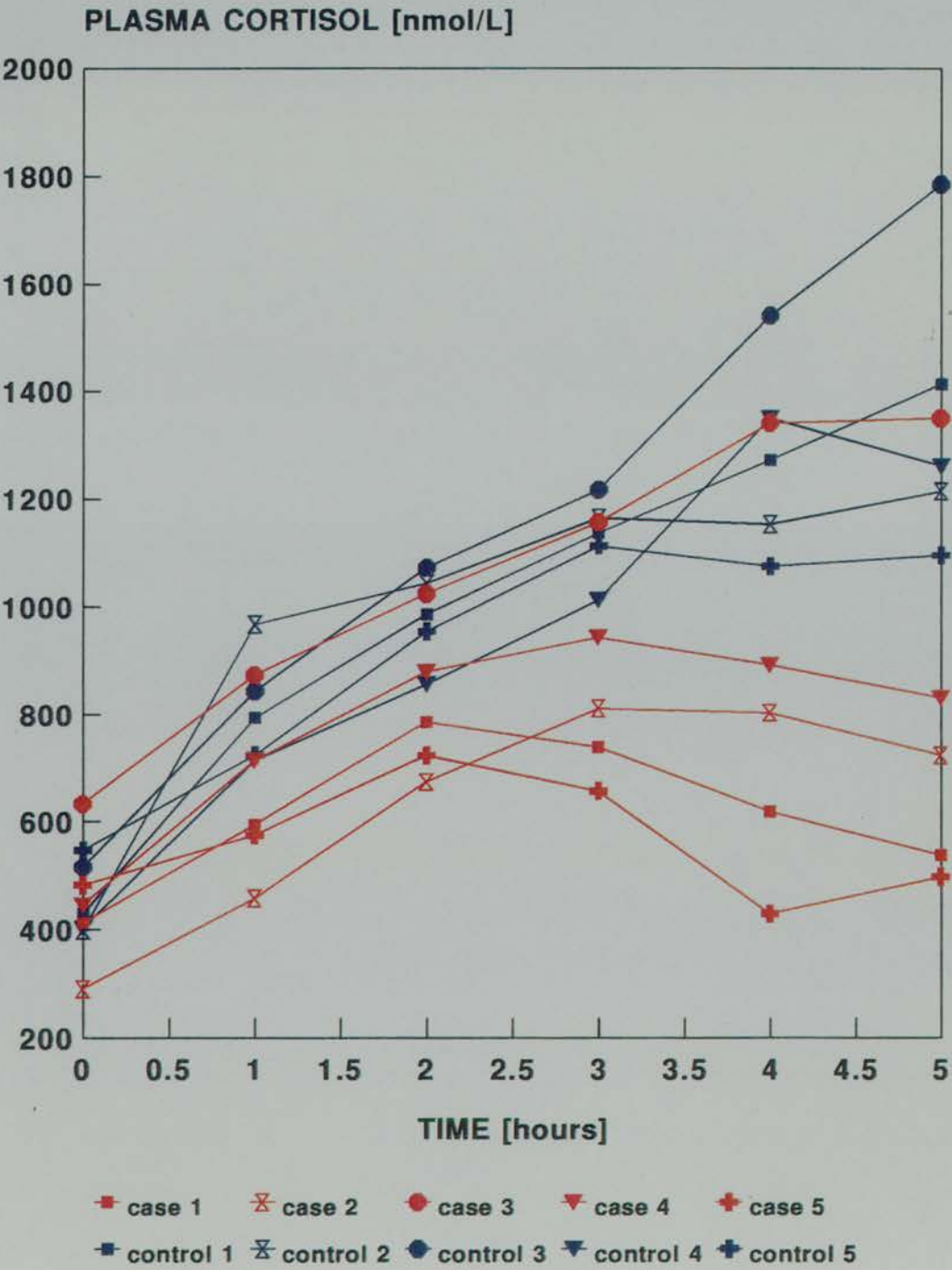
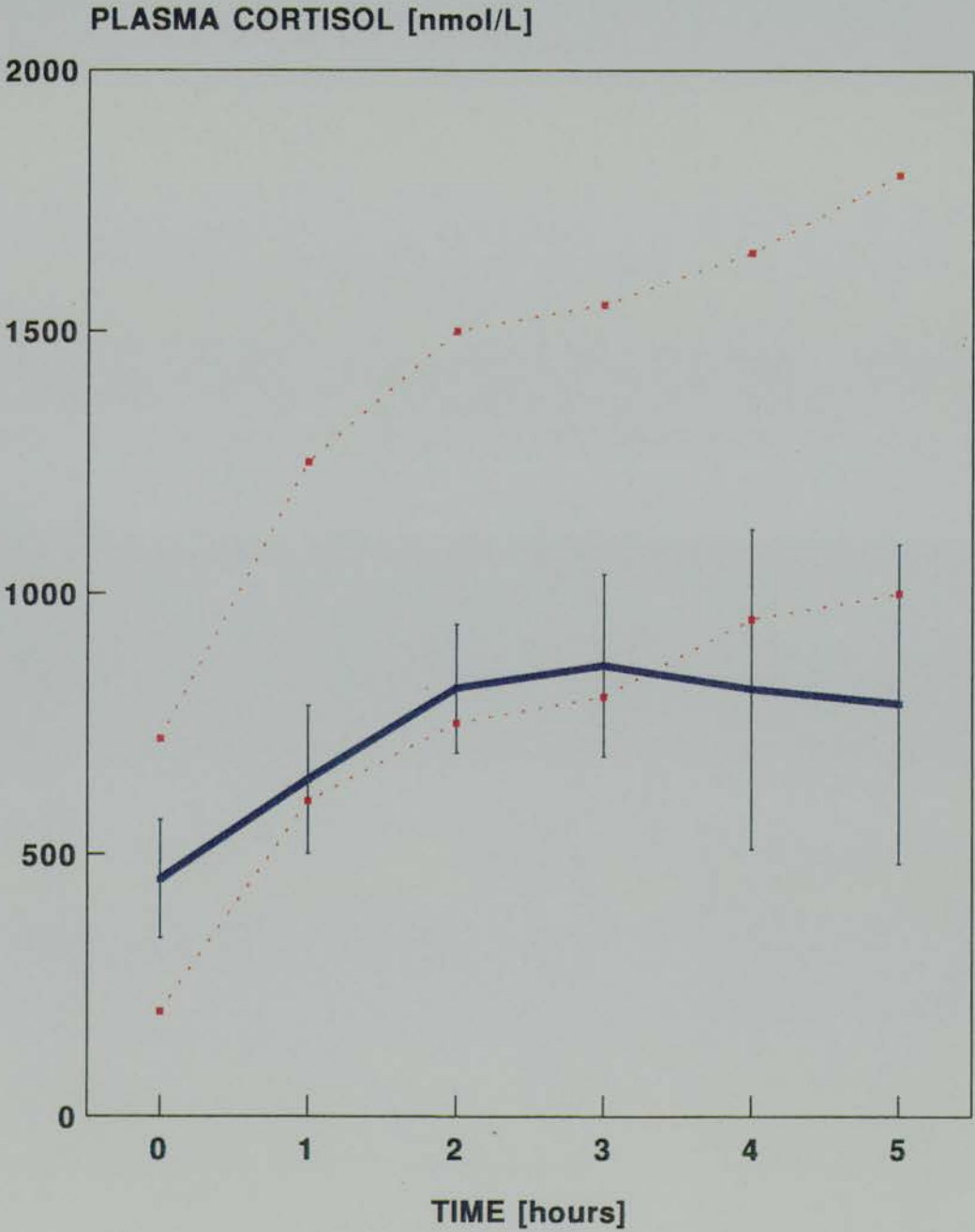
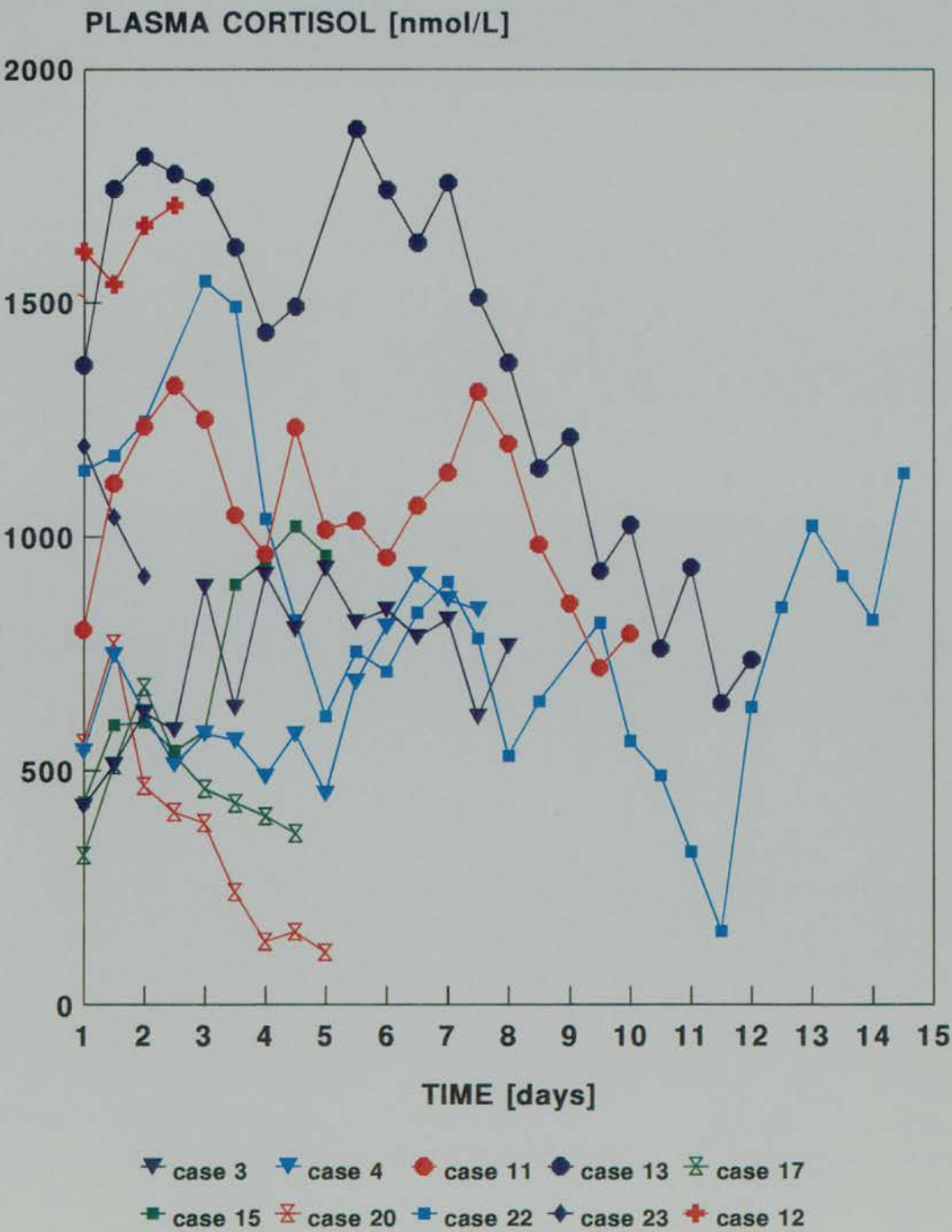


FIG 4.18. MEAN [95% CI] PLASMA CORTISOL RESPONSE TO
DEPOT ACTH STIMULATION.



I cases [mean + 95% CI] - - - limit of normal range

FIG 4.19. TWICE DAILY PLASMA CORTISOL CONCENTRATION
IN SEPTIC SHOCK



MISSING DATA - CASES 12 AND 15

(5) Discussion

Plasma Cortisol Concentration and Severity of Illness

Cortisol was measured on blood taken within 2 hours of admission to the ICU. This ensured that all patients were investigated at a uniform time during their ICU stay. In view of the loss of circadian rhythm of cortisol concentration, this was felt to be more important than measurement at a fixed time of day, and is supported by the lack of a correlation between the time of ICU admission and the cortisol concentration.

The distribution of plasma cortisol concentration was non-parametric, with a long tail of high concentrations. Logarithmic transformation produced a parametric distribution. Eighteen percent of patients had a concentration greater than 1500 nmol/L and 7% had a concentration greater than 2000 nmol/L. These cortisol concentrations are well above the normal range for healthy controls, the upper limit for which is quoted as 720 nmol/L at 0800h (Ismail, 1981). Only 92 patients [35%] had an admission plasma cortisol concentration below 720 nmol/L. High cortisol concentrations have been found previously in acutely ill and intensive care patients [Table 4.15]. A number of the papers listed below have been published since this study began.

The cortisol concentrations in this study [mean = 1012 nmol/L (SD = 621) and median = 940 nmol/L (range 100 - 3790)] are similar to those found in previous studies. However, meaningful comparison is difficult without a method

of comparing the patients in each of the studies. In the majority of the studies listed in Table 4.15, little information is given about the severity of illness or primary diagnoses of patients studied. The mean APACHE II scores in the patients studied by Span et al and those studied by Journey et al were 19 and 18.5 respectively. These are similar to the mean score in this study, suggesting that the severity of illness was similar, although the range of illnesses may have differed.

Table 4.15. Previous studies looking at cortisol concentration in acutely ill and intensive care patients.

REFERENCE	NUMBER OF CASES	PRINCIPAL DIAGNOSES	CORTISOL [nmol/L]
Span et al, 1992	159	General ICU	600 [SD = 280]*
Journey et al, 1987	70	General ICU	988 [SD = 265]*
Schein et al, 1990	37	Septic shock	1430 [520 - 9000]
Patel et al, 1991	50	General medical	665 [288 - 1585]
Frayn et al, 1983	51	Hip fracture	910 [350 - 2310]
Sainsbury et al, 1981	30	General ICU	1200 [130 - 4920]
Feibel et al, 1983	65	Stroke	552 [240 - 1000]
Ross et al, 1991	6	General ICU	567 [SD = 57]*
Sibbald et al, 1977	26	Septic shock	710 [SD = 390]*
Stoner et al, 1979	270	Acute trauma	820 [200 - 1500]
Jensen et al, 1988	18	Myocardial infarct	750 [SD = 250]*
Drucker et al, 1985	40	General medical	980 [212 - 8430]

* = mean [SD], otherwise median [range].

One of the aims of this work was to produce normal ranges of

cortisol concentration. A normal range is usually two standard deviations about the mean of the concentration of a given substance in a reference population, usually healthy controls. It follows that in 95% of cases, if the subject under study is comparable to the reference population, and has a normally functioning endocrine system, the concentration will fall within the normal range. It is more difficult to define a normal hormone concentration with which to compare an individual ill patient. Strictly, this would require a large group of subjects who were suffering from the same illness, and in whom the illness was equally severe in each case. Clearly, this is not possible.

Seyle's General Adaption Theory suggests that different stresses produce similar responses from the adrenocortical system. It follows that different illnesses which induce the same degree of stress should produce comparable adrenocortical responses. If this is the case, then a normal range of cortisol concentration in ill subjects will depend more upon the degree of stress or illness, than upon the nature of the illness itself. The degree of stress to which a patient is subjected is often equated with the degree to which the homeostasis of the patient is deranged. This is the basis of measuring illness severity by the APACHE system [Knaus et al, 1985]. The positive correlation between admission APACHE II scores and admission cortisol levels in this study supports Seyle's General Adaption Theory. The more marked the stress, measured by APACHE II scores, the higher the plasma cortisol concentration.

Only 3 studies have commented on a correlation between cortisol concentration and measures of illness severity. Stoner et al (1979) found a positive correlation between plasma cortisol concentration and the Injury Severity Score in trauma patients investigated within 8 hours of injury. Vaughan et al [1982] studied patients who had suffered major burns, and found a positive correlation between burn size and plasma cortisol concentration. Span et al [1992] investigated 159 ICU patients and found a clear correlation of plasma cortisol concentration with APACHE II scores.

Given the strength of the association between cortisol concentrations and severity of illness, any attempt to define a normal range for plasma cortisol concentration during illness, must take illness severity into account. APACHE II scores are the most widely used and validated measure of illness severity. The admission cortisol data were therefore presented as a series of normal ranges for differing degrees of illness severity, based on bands of APACHE II scores. There is much overlap of cortisol concentration at all APACHE II scores, and it therefore made little sense to have more than 3 bands. In order that each group contained sufficient measurements to produce a meaningful normal range, the following bands were chosen: < 16, 16-24, and > 24.

Whilst the normal ranges of all the bands do overlap, the mean cortisol concentrations do differ significantly. The differences between the 95% ranges for APACHE II scores of <16 and >24 are important, the lower limits being 200 nmol/L

and 400 nmol/L respectively. A severely ill patient with a high APACHE II score would not be expected to have a plasma cortisol level of less than 400 nmol/L, whereas this would be acceptable in a normal control or a less severely ill patient. A concentration outwith the normal range does not necessarily indicate an abnormality of adrenocortical function, but may require further investigation.

The overall correlation between plasma cortisol concentration and mortality was as expected. As plasma cortisol concentration increased, so did mortality. The mean admission cortisol concentration in survivors was significantly lower than in patients who died. Previous studies have produced conflicting results. Journey et al (1987) produced similar results to this study, but Schein et al (1990) found no correlation between plasma cortisol concentration and mortality. However, the latter study was confined to patients with septic shock all of whom were very ill and had a narrow range of APACHE II scores. There was no correlation between baseline cortisol concentration and APACHE II scores in the subgroup of 32 septic shock patients who underwent standard ACTH stimulation tests in this study.

Whilst in general mortality does increase with increasing cortisol concentration, patients with low cortisol concentrations for their severity of illness also have a high mortality. In the upper APACHE II score band [> 24] the mortality among patients with the lowest cortisol concentrations [< 500 nmol/L] was significantly higher than the mortality of patients in the same band who had higher corti-

sol levels [500-1000 nmol/L]. Similarly, in the less severely ill patients, cortisol concentrations below the normal range for their APACHE II score were also associated with a higher than expected mortality. This association between high mortality and low cortisol in very severely ill patients has not been reported previously, and illustrates the importance of interpreting cortisol concentrations in the context of illness severity. The implications of these findings for clinical trials of cortisol replacement in critical illness are discussed in Chapter 8.

Higher admission cortisol concentrations were associated with acute renal failure. However, the association disappeared when a correction was made for differing non-renal APACHE II scores, suggesting that the association was due to more severe illness in patients with acute renal failure.

Research in animals has shown that hypoxia is a profound stimulus to cortisol secretion (Raff et al, 1981), and prolonged hypoxia in human volunteers leads to a persistent 50% rise in cortisol concentration (Hale et al, 1957). In this study there was no correlation between arterial partial pressure of oxygen [pO_2] and plasma cortisol concentration. However, hypoxia was only one of a large number of physiological stresses affecting the patients studied, and any independent effect on cortisol concentration was therefore likely to be lost. Furthermore, a single pO_2 measurement is unlikely to accurately represent the overall exposure to hypoxia over the preceding hours. Indeed, the admission arterial blood gas sample is often not taken until the

patient has been intubated and ventilated, and would probably bear little resemblance to a pre-admission measurement. Admission cortisol concentration did not correlate with length of illness prior to ICU admission, nor did post-anaesthetic patients have higher levels than non-anaesthetic cases. Both these findings are in agreement with previous work (Span et al, 1992).

Serial Plasma Cortisol Measurements

There were two reasons for looking at serial measurements of plasma cortisol. Firstly, to determine how much plasma cortisol concentration varied over the short term following ICU admission. The lack of diurnal variation has already been demonstrated, but variability in cortisol concentration due to evolution of illness or medical interventions might still undermine the value of a single measurement. Secondly, if outcome in patients suffering from septic shock was in some way shown to be related to admission plasma cortisol concentration, then monitoring cortisol levels until death or recovery might reveal a consistent pattern depending upon the outcome.

The variation in cortisol measurements over the first 48 hours of the study was no greater than would be expected on the basis of the intra-assay coefficients of variation for high cortisol concentrations demonstrated in Chapter 2. This suggests that a single admission cortisol measurement is a reasonable valid index of the cortisol concentration during the first 48 hours after admission.

Interpretation of the trends in cortisol concentration over the whole admission is difficult. In 3 of the 6 nonsurvivors cortisol concentration fell steadily to very low levels prior to death, one patient requiring cortisol replacement. However, in the remainder of nonsurvivors, no such pattern was seen. Cortisol concentration remained relatively constant in the survivors, although a tendency to fall from very high to more normal levels was seen at recovery. No similar studies have been published since the advent of modern intensive care. However, as detailed in Chapter 1, there is some useful early work. Jennings (1951 and 1952) followed the circulating eosinophil counts of 50 severely ill patients, all of whom died. In 47 of these, the eosinophil count fell to zero or near zero prior to death, probably indicating a strong adrenocortical reaction. Sandberg et al (1956) studied 17-hydroxycorticosteroids in dying patients. In the great majority of these, the plasma steroids were elevated. Done et al (1958) investigated 64 severely ill adults a few minutes after death. Plasma corticosteroids were raised in the majority of cases. Interestingly, in a small group of 11 cases who had suffered prolonged hypotension or apnoea, no rise in plasma corticosteroids had taken place.

Standard ACTH Stimulation Tests

Interpretation of the results of ACTH stimulation tests in critical illness is difficult and only limited conclusions can be drawn. In the absence of information about free

cortisol concentrations, and for that matter tissue cortisol requirements, the ACTH stimulation test cannot be used to identify adrenocortical insufficiency in the way that it can in health. All work validating ACTH stimulation tests has been carried out in healthy controls (Wood et al, 1965; Crowley et al, 1991) or in out-patient populations (Lindholm and Kellet, 1987; Stewart et al, 1988). Moreover, even if relative adrenocortical insufficiency did occur during severe illness, there is no clinical gold-standard for its diagnosis against which ACTH stimulation tests could be validated.

ACTH stimulation tests cannot provide any information about the sufficiency of adrenocortical function during critical illness. However, as was discussed in Chapter 1, they can be used to determine any relationship between the cortisol response to ACTH and severity of illness or mortality. In this study, the cortisol response to a standard dose of ACTH was very variable, ranging from no response at all to increases of 1000 nmol/L. The interpretation of this varied response is also difficult. In healthy controls, a poor response can be interpreted as suggesting impairment of adrenocortical responsiveness to ACTH e.g. following prolonged steroid therapy. However, in critically ill patients interpretation is complicated by the fact that, in the majority of cases, the baseline cortisol concentration is high. In healthy controls, the cortisol increment following ACTH stimulation is independent of the basal cortisol concentration (Kukreja and Williams et al, 1981; May and Carey, 1985;

Dickstein et al, 1991), but this may not be the case in patients with markedly raised cortisol concentrations.

In this study, there was a relatively minor overall tendency for the cortisol increment following ACTH stimulation to decrease as basal cortisol concentration increased. However, this was only apparent in patients who subsequently died, and was only a modest effect in the group as a whole. Previous studies of ACTH stimulation tests during severe illness [Table 4.16] found that the cortisol increment remained constant as basal cortisol increased. Indeed, in the studies by Sibbald et al (1977) and Journey et al (1987), those patients with the highest basal cortisol concentrations had the greatest response to ACTH. In this thesis, many patients with high baseline cortisol concentrations did produce a large cortisol increment following ACTH. Overall, it appears that a response to ACTH should still be expected in individuals with high cortisol concentrations. Apparent impairment of the adrenocortical response to ACTH may therefore be a valid finding requiring interpretation and further study.

In none of the previous studies was the response to ACTH reported in relation to severity of illness or mortality. In this study, the basal cortisol concentration did not vary with severity of illness, but the mean cortisol increment following ACTH stimulation fell as the APACHE II score increased. Furthermore, in both non-shocked and septic shock cases, the fall in cortisol increment with increasing APACHE II score was especially marked in patients who subsequently

died. Indeed, non-survivors as a whole had lower increments than survivors. These results indicate that the variability in response to a standard ACTH test in critical illness has clinical relevance.

Table 4.16. Previous studies looking at the cortisol response to standard ACTH stimulation tests during illness.

REFERENCE	NUMBER OF CASES	BASAL CORTISOL mean [range]	CORTISOL INCREMENT mean [range]
Sibbald et al, 1977	28	820 [210 - 2420]	450 [0 - 2000]
Jurney et al, 1981	70	980 [226 - 2184]	560 [0 - 1200]
Patel et al, 1991	50	706 [288 - 1585]	374 [10 - 740]

In both septic shock and non-shocked patients, a plasma cortisol increment of less than 250 nmol/L was associated with an increased mortality, irrespective of the APACHE II score. It is, of course, unclear why a poor response to ACTH is associated with a high mortality. It may simply be a particularly sensitive indirect index of illness severity. Function of most organs is impaired to a greater or lesser extent during critical illness. There is no reason to suspect that the adrenal cortex should be an exception. Indeed, as was detailed in Chapter 1, there is much evidence of impairment of adrenocortical function in animal models of shock and sepsis. The results of this study suggest that in

certain patients critical illness is associated with an impaired response to ACTH. The implications of the association between the response to a standard ACTH stimulation test and mortality for clinical trials of cortisol replacement in critical illness will be discussed in Chapter 8.

Only one patient was as suffering from adrenocortical insufficiency by standard criteria, with a basal cortisol concentration of 220 nmol/L and an increment of only 93 nmol/L following ACTH stimulation. This patient was suffering from severe Gram-negative sepsis and died despite cortisol replacement therapy. However, such clear adrenal insufficiency is rare. None of the 50 patients investigated by Patel et al had stimulated cortisol concentrations below 550 nmol/l. Two patients investigated by Journey et al had stimulated cortisol concentrations below 400 nmol/l. Both were suffering from sepsis, recovered with cortisol replacement therapy, and had a normal response to a repeated ACTH stimulation test on recovery. Sibbald et al identified five patients suffering from sepsis syndrome with stimulated cortisol concentrations of less than 490 nmol/L, and with cortisol increments of less than 100 nmol/L.

The septic shock cases had lower basal cortisol concentrations than the non-shocked cases, despite a greater mean severity of illness according to APACHE II scores. The main difference in management was the more frequent use of adrenaline infusions for inotropic support in the septic shock cases. However, intravenous adrenaline is reported to increase cortisol concentrations (Tilders et al, 1985). Within

the septic shock group, those patients with proven Gram-negative sepsis had lower cortisol increments following ACTH stimulation than patients with Gram-positive infection. It has been shown previously that in septic shock, patients with Gram-negative infection have lower baseline plasma cortisol levels than those with Gram-positive infection (Schein et al, 1990; Khosla et al, 1989). The findings in this study are consistent with the experimental evidence linking impaired adrenocortical function with Gram-negative infection reviewed in Chapter 1.

In summary, it appears that the cortisol response to ACTH is fairly well maintained in critical illness, despite high basal cortisol concentrations. However, a proportion of patients with high basal cortisol concentrations have a poor cortisol increment. This is most frequently seen in patients suffering from septic shock, and is associated with a high mortality.

Low Dose ACTH Stimulation Tests

As was discussed in Chapter 3, low dose corticotropin stimulation tests have been shown to be more sensitive to steroid suppression of adrenocortical function than standard ACTH stimulation tests (Dickstein et al, 1991). There is no published data on the cortisol response to low dose ACTH during critical illness. The purpose of using low dose tests in this study was to determine whether the adrenal cortex was as sensitive to low doses of ACTH during illness as it is during health.

The cortisol response to low dose ACTH in control cases was similar to previously reported results (Graybeal and Fang, 1985; Crowley et al, 1991; Dickstein et al, 1991). The response in the non-shocked ICU patients did not differ significantly from that of the control cases, despite the higher basal cortisol concentration in these patients. However, the response to the low dose ACTH in the septic shock cases was significantly smaller than that in the non-shocked cases. These findings suggest a normal sensitivity to ACTH in non-shocked critically ill patients, but a reduced sensitivity to ACTH or decreased secretory capacity in septic shock patients. This is consistent with the results of standard ACTH stimulation tests in septic shock cases.

Depot ACTH Stimulation Tests

There are no published data on the cortisol response to depot ACTH tests during critical illness or septic shock. The purpose of these tests in this study was to assess the adrenocortical reserve in septic shock patients. The cortisol response of the control cases was within the expected normal range, but the response in the septic shock patients was less marked and less well maintained. The mean cortisol concentration fell below the lower limit of the normal range at 4 and 5 hours after the injection, suggesting that these patients may have had a limited adrenocortical reserve capacity. Unfortunately no tests were performed in non-shocked critically ill patients for comparison.

Repeat Standard ACTH Stimulation Tests on Recovery

The mean cortisol increment in the 10 septic shock patients investigated following recovery was higher than the mean increment in the original tests, but this did not reach statistical significance. These data are difficult to interpret, but suggest that in the patients studied there was no significant difference between adrenocortical responsiveness to a standard dose of ACTH during illness and following recovery. However, it should be noted that the cases investigated had a good response to ACTH during their illness. The majority of patients in whom adrenocortical responsiveness to ACTH was impaired during illness died.

There are isolated reports of patients suffering from sepsis or septic shock, with no history of steroid treatment during or prior to their illness, who do not respond to ACTH during their illness but regain normal function on recovery (Jacobs and Nabarro, 1969; Journey et al, 1987; Varma et al, 1990). The significance of such isolated reports is difficult to assess.

(6) Conclusions

The aims of this study were limited. No attempt was made to determine whether or not relative adrenocortical insufficiency does occur in critical illness. However, a number of interesting findings have emerged. The main conclusions are as follows:

- 1) The majority of critically ill patients have plasma

cortisol concentrations above the upper limit of normal in health. In keeping with Seyle's General Adaption Theory, plasma cortisol concentration correlates with severity of illness. The normal ranges of plasma cortisol concentration, defined as population mean \pm 2 standard deviations, differ according to the severity of illness. A plasma cortisol concentration above 200 nmol/L would be within the 95% range for a moderately ill patient (APACHE II score <16) whereas a concentration of under 400 nmol/L would be below the expected range in a severely ill patient (APACHE II score >24).

2) The relationship between mortality and plasma cortisol concentration is not linear. Rather, this study suggests a J-shaped relationship. In other words, mortality increases as cortisol concentration increases above normal, but low cortisol concentrations are also associated with a higher than expected mortality. The implications of this finding for clinical trials of cortisol replacement during critical illness are discussed in Chapter 8.

3) The cortisol response to a standard ACTH stimulation test varies between -100 and 1000 nmol/L in critically ill patients. In patients who subsequently survive, the cortisol response is maintained despite very high basal cortisol concentrations. In patients who subsequently die, the cortisol response tends to fall as basal plasma cortisol concentration increases. Overall, there is a negative correlation between the cortisol response to a standard ACTH stimulation

test and APACHE II scores.

4) The cortisol response to a standard ACTH stimulation test was related to mortality. The mortality among patients with cortisol responses of less than 250 nmol/l was 76% [95% CI, 55-91] compared with 31% (95% CI, 19-45) mortality among patients with cortisol responses greater than 250 nmol/L. Although the former patients tended to have higher APACHE II scores, the same mortality trend was present in the three standard APACHE II score groups. The implications of this finding for clinical trials of cortisol replacement during critical illness are discussed in Chapter 8.

5) There was evidence of impairment of adrenocortical function in some critically ill patients, particularly those suffering from septic shock. Septic shock patients had lower cortisol responses to standard and low dose ACTH stimulation tests than critically ill controls and the cortisol response to depot ACTH was not maintained in septic shock patients.

Directions for Future Research

The studies outlined in this chapter have helped define the critically ill patients in whom pilot studies of cortisol replacement could be most efficiently performed. This will be discussed in detail in Chapter 8.

The apparent impairment of adrenocortical function in some critically ill patients requires further study. Post mortem studies of adrenal gland pathology comparing patients with

good responses to standard ACTH stimulation tests with patients with poor or absent responses would be worthwhile. Although not falling within the remit of this thesis, the question of whether high mortality among patients with apparent impairment of adrenocortical function is due to cortisol insufficiency could be further investigated. Do these patients have lower circulating free cortisol concentrations? In other words, is tissue availability of cortisol reduced? If not, then the argument that their high mortality is due to impaired adrenocortical function would be difficult to maintain. Measurement of free cortisol concentration and the concentrations of cortisol binding globulin and albumin during critical illness would therefore be interesting. However, since we do not know what the range of tissue requirement for free cortisol during critical illness is likely to be, it does not necessarily follow that a high measured concentration is sufficient. The question of whether critically ill patients actually benefit from cortisol replacement is easier to answer and is of far more practical importance.

Chapter 5

Historical Background, Structure, Physiology and Investigation of Thyroid Function

- (1) Historical Background
 - (2) Anatomy, Biochemistry and Physiology of the Thyroid Gland
 - (3) Investigation of Thyroid Function
 - (4) Factors Confounding the Interpretation of Thyroid Function Tests
During Critical Illness
-

(1) Historical Background

References to the thyroid gland and thyroid diseases can be found throughout ancient medical literature. Thyroid enlargement was described by the Greeks. Galen used the term *bronchocele*, meaning hernia of the windpipe. Pliny introduced the Latin term *tumid gutter*, meaning swollen throat, which subsequently evolved into the French *goitre*, the English *goitre*, and the American *goiter*. However, it was not until the 1700s that it was established that the thyroid was a single glandular organ (Thompson, 1970).

There was much speculation regarding the function of the gland. Wharton suggested that the gland was intended to "round out and beautify the neck by filling the vacant spaces about the larynx particularly in females to whom for this reason a larger gland has been assigned." Vercelloni argued that "the thyroid is a bag of worms" which occasionally cross, for digestive purposes, into the oesophagus (Vercelloni, 1711).

Ancient Chinese physicians are said to have treated endemic goitre with seaweed (Matovinovic, 1958). However, it was not until the late nine-

teenth century that the functional role of the thyroid and parathyroid glands was elucidated. Much work had been done on experimental thyroidectomy, but it was not until 1896 that Vassale and Generali separated the syndrome of myxoedema from that of tetany. At about the same time it was found that injection of thyroid extract (Murray, 1891) or feeding lightly cooked sheep thyroid (Vermeulen, 1893) would relieve the signs and symptoms of myxoedema.

The first descriptions of hyperthyroidism were given by Parry (1825) and Graves (1835), and shortly afterwards von Basedow (1840) described the ophthalmic manifestations.

The association between iodine and the thyroid gland was made in 1896 (Baumann, 1896) and in 1915 L-thyroxine was crystallised from thyroid extract (Kendall, 1915). It was later discovered that a second compound with only three iodine atoms, 3,5,3'-triiodothyronine, was present in the thyroid gland and plasma (Gross, 1954). It was subsequently proven that T3 was produced by peripheral monodeiodination of T4, and that it was the main physiologically active form of the hormone (Braverman, 1970).

The concept of pituitary control of the thyroid gland stretches back to the observation (Niepce, 1851) that the pituitary gland was markedly enlarged in cretins, and the work demonstrating hypertrophy of the anterior pituitary after thyroidectomy in the rabbit (Rogowitsch, 1889). This concept was confirmed by the demonstration that ablation of the tadpole pituitary resulted in thyroid atrophy and prevented metamorphosis (Smith, 1915) and that the atrophied thyroid gland could be made to hypertrophy by injections of beef anterior pituitary extracts (Smith, 1922). Shortly thereafter the negative feedback control system of the pituitary-thyroid axis was defined (Aron, 1931). More recently the structure of the pituitary TSH and the control of its secretion by the hypothalamic thyrotropin releasing hormone [TRH] has been established.

(2) Anatomy, Biochemistry and Physiology of the Thyroid Gland

The normal adult thyroid gland consists of two lobes connected by an isthmus and weighs about 15 to 25g. The vascular supply is derived from the superior and inferior thyroidal arteries, and the gland contains a rich lymphatic network. The thyroid gland is divided into lobules, each of which contains 20 to 40 follicles. The follicles are lined by epithe-

lial cells which surround central deposits of colloid. It is estimated that the adult thyroid gland contains approximately three million follicles. The colloid serves as a store for thyroglobulin, iodine and thyroid hormones.

The thyroid hormones, L-thyroxine [T₄] and L-triiodothyronine [T₃], are synthesised in specialised cells of the thyroid gland. Iodine is essential for the synthesis of thyroid hormones. T₄ contains 4 iodine atoms per molecule and is 66% iodine by weight. T₃ is 58% iodine by weight with 3 atoms per molecule. Iodine is actively transported into the thyroid gland and concentrations 30 times greater than serum iodine are reached. This is one of the rate limiting steps in thyroid hormone synthesis and is under the control of TSH. Within the thyroid cells iodine ions are rapidly "organified" or converted to iodine and bound to one of the 110 tyrosine residues of thyroglobulin molecules synthesised by the cell.

Iodothyronines are formed by coupling of iodotyrosines on neighbouring thyroglobulin molecules. The coupling process is enzyme dependent and is controlled by TSH. T₃ is formed by the coupling of a monoiodotyrosine with a diiodotyrosine, and T₄ is formed by coupling of two diiodotyrosine molecules. The newly formed hormone remains part of the thyroglobulin molecule, and is stored in the colloid. Ordinarily, less than 1% of stored hormone turns over each day.

Regulation of Thyroid Function

Like the gonads and adrenal cortex, the thyroid interacts with the hypothalamus and the pituitary gland in a classic type of feedback control. Thyrotropin releasing hormone [TRH] is a modified tripeptide [pyroglutamyl-histidyl-proline amide] which is synthesised by peptidergic neurones in the supraoptic and paraventricular nuclei of the hypothalamus. It is transported to and stored in the median eminence. From here it enters the hypophyseal portal venous system and traverses the pituitary stalk. Thyrotropic and lactotropic cells contain specific saturable receptors to which TRH binds and stimulates adenylate cyclase. Under the influence of TRH, secretion of TSH is promptly stimulated and enhanced TSH synthesis follows. The response to TRH requires extracellular calcium and oxidative phosphorylation within the pituitary, but it is unclear whether cyclic AMP or translocations of calcium mediate the response.

Regulation of TSH secretion results from an interaction, possibly entirely at the level of the thyrotropic cell, between stimulation of synthesis and release by TRH and inhibition by thyroid hormones. Inhibition of TSH synthesis and release by thyroid hormones is not merely a direct antagonism of TRH, since it can be observed in the experimental absence of TRH. Moreover, the degree of hypothyroidism that results from destruction of the appropriate areas of the hypothalamus is less severe than that which follows hypophysectomy. In the former circumstance, residual TSH secretion can be varied by changing the concentration of thyroid hormones in the blood. Thus, thyroid hormones mediate the feedback regulation of TSH secretion and TRH determines its set point. There is no convincing evidence that thyroid hormones directly modify the secretion of TRH.

TSH is a glycoprotein made up of two subunits. The alpha subunit is identical to the alpha subunits of follicle stimulating hormone [FSH], luteinising hormone [LH] and human chorionic gonadotropin [hCG]. Each hormone differs in its beta subunit. Production of the beta subunit is the rate limiting step in the synthesis of TSH. The mechanism of negative feedback on TSH production by thyroid hormones is incompletely understood. Briefly, although in peripheral tissues T₄ is probably a prohormone of T₃, T₄ appears to have an important role in regulation of TSH at the pituitary level. Intra-pituitary T₃ is derived from circulating T₄ which is deiodinated within the pituitary cells. Thus TSH will rise in response to decreased serum T₄ levels despite normal or elevated T₃ levels (Wehmann and Nisula, 1984).

Numerous factors modulate TSH secretion. A number of substances appear to play a role as TSH inhibitory factors. Infusions of dopamine decrease circulating TSH levels in euthyroid and hypothyroid subjects (Delitala, 1977; Kaptein, 1980), and reduce the TSH response to TRH (Besses et al, 1975). Dopamine antagonists such as metoclopramide (Scanlon et al, 1977) and domperidone (Pourmand et al, 1980) both promptly increase TSH levels. Domperidone does not cross the blood-brain barrier suggesting that dopaminergic inhibition of TSH is exerted at the level of the pituitary gland. Dopamine is found in high concentration in the portal blood and dopamine receptors are present on thyrotrophs, suggesting that dopamine is an important physiological inhibitor of TSH secretion. Somatostatin (Vale et al, 1974) and cholecystokinin (Morley et al, 1979) also appear to play similar roles.

Glucocorticoids exert multiple effects on the H-P-T axis. It has been suggested that the circadian rhythm of TSH secretion is the result of an inhibitory action of plasma cortisol (Nicoloff et al, 1970). TSH concentrations peak in early hours of the morning and are lowest at midday. Pharmacological doses of steroids inhibit basal TSH secretion, the nocturnal surge in TSH secretion, and the response to TRH (Re et al, 1976). TSH levels are low in Cushing's syndrome (Van Cauter et al, 1974) and high in Addison's disease, falling to normal on steroid replacement (Topliss et al, 1980). It is thought that steroids influence the H-P-T axis at both the pituitary and hypothalamic levels (Morley, 1981).

Men have lower TSH responses to TRH than women (Sawin et al, 1978). Androgens decrease the TSH response to TRH in men with hypogonadism (Morley et al, 1981) and are thought to be physiological inhibitors of TSH secretion. Oestrogens enhance TRH receptor density on the surface of the thyrotropic cell and increase the TSH response to TRH. Endogenous opiates probably have little effect on TSH secretion (Morley, 1981). High concentrations of exogenous opiates have an inhibitory effect on TSH secretion (Morley et al, 1980). Catecholamines stimulate TSH secretion via an effect on hypothalamic TRH release (Montoya et al, 1979).

Psychological stress has a weak stimulatory effect on thyroid hormone levels. Falconer and Hetzel (1964) showed that a dog barking at sheep led to a rapid increase in thyroid hormone secretion associated with increased circulating TSH. Similar results have been found in humans exposed to mock battle conditions (Levi, 1972). Temperature changes alter thyroid function. Exposure to cold leads to enhanced TSH secretion in the rat and other animals (D'Angelo, 1960). This response is mediated via the hypothalamus. In humans the response of TSH to cold stress is demonstrable but small (Goldstein-Golai et al, 1970). Heat exposure leads to a decline in serum TSH and release of thyroid hormones in humans (O'Malley et al, 1980).

TSH is the major regulator of thyroid structure and function. However, there is some activity of thyroid cells in the absence of TSH, although this is not sufficient to maintain a euthyroid state. There is also a degree of thyroid autoregulation which is independent of TSH. This is most evident in the regulation of uptake of iodide. However, it is unclear to what extent autoregulation is an important determinant of thyroid hormone concentration. It appears to act to maintain relatively constant thyroid hormone stores.

TSH is the principal controller of thyroid gland function. Removal of TSH stimulation results in hypovascularity and atrophy of the gland. TSH acts via cyclic AMP dependent stimulation of protein kinases which in turn stimulate the steps in thyroid hormone synthesis. TSH appears to enhance essentially all processes leading to the synthesis and secretion of thyroid hormones. Neural regulation of thyroid gland function probably exists but is not considered to be important. Adrenergic and peptidergic innervation increase hormone secretion and cholinergic innervation decreases hormone secretion.

Thyroid Hormone Circulation and Protein Binding

The concentration of thyroid hormones in the blood are determined to a great extent by their association with thyroid hormone-binding proteins. Interpretation of circulating thyroid hormone concentrations in the absence of information on protein binding is therefore difficult. Approximately 120 nmol of T₄ and 45 nmol of T₃ are secreted daily. T₄ is 99.97% protein bound in the plasma, and T₃ is 99.7% bound. Approximately 75% of the hormones are bound to thyroxine-binding globulin [TBG], 15% to thyroxine-binding pre-albumin [TBPA] and 10% to albumin. TBPA binds T₄, but only very small amounts of T₃. This very high degree of protein binding has several important consequences. The plasma has a large capacity to store hormone, acting as a buffer against fluctuations in blood levels. Very little hormone is lost through renal glomerular filtration of free hormone. Changes in the levels of binding proteins will alter the amount of free hormone available for cellular uptake. T₄ is more highly bound than T₃, and has a smaller volume of distribution. The half life of T₄ is about 1 week compared to 1 day for T₃. Albumin and TBPA are present in the blood in much higher concentration than TBG and have a greater capacity than TBG to bind T₄. However, the affinity of TBG for binding T₄ is several orders of magnitude greater than albumin or TBPA, although only about 30% of binding sites for T₄ on TBG are occupied. TBG is a glycoprotein with a molecular weight of approximately 54,000 and a half-life of five days. TBG concentration is altered in certain clinical circumstances and may lead to increased or decreased total thyroxine levels. Factors increasing TBG concentration include oestrogens, pregnancy, hepatitis and porphyria. Low TBG levels are associated with androgens, cirrhosis, nephrosis and large doses of steroids.

Metabolism of Thyroid Hormones

Thyroid hormones undergo peripheral metabolism following secretion. T₄ is metabolised by sequential removal of its iodine atoms. This process is termed deiodination. Although all tissues are able to do this, most T₄ is metabolised in the liver and kidney. Removing either iodine atom from the outer ring [the 3' or 5' positions] of T₄ yields T₃. By convention this is referred to 5'-deiodination. T₃ has greater metabolic activity and so this process represents activation of T₄. Removing either iodine atom from the inner ring of T₄ yields 3,3',5'-triiodothyronine, known as reverse T₃ [rT₃]. Reverse T₃ has little or no metabolic activity, and so 5-deiodination represents inactivation of T₄. About 30% of the T₄ secreted each day is deiodinated to produce T₃; this accounts for 85% of the T₃ produced each day. Deiodination is effected by specific enzymes, three of which have been identified (Leonard, 1990). Type 1 deiodinase effects both 5 and 5' deiodination and is inhibited by 6-propylthiouracil. Type I deiodinase is found mainly in the liver, kidney and thyroid. Type II deiodinase is present in brain, pituitary and brown adipose tissue and active in only 5'-deiodination. Type III deiodinase is found in brain, skin and placenta deiodinates at both locations. Both type II and type II enzymes are insensitive to propylthiouracil.

Thyroid hormones influence function of nearly all organs and tissues. The extent of the influence is proportional to the degree of nuclear receptor binding of T₃ in the tissue. High T₃ binding is found in thyroid responsive tissues such as liver and kidney, whereas low binding is found in tissues such as spleen in which thyroid hormones exert less powerful effects. Entry of T₃ across the cell membrane and into the cytoplasm is by simple diffusion, as is T₃ entry into the nucleus. Nuclear receptors have been characterised and are tightly bound to chromatin. Although T₄ binds to nuclear receptors, 90% of nuclear thyroid hormone is T₃. In many respects T₄ is simply a prohormone of T₃. The marked effects of T₃ on development, growth and function of the organism are subserved in large part via regulation of gene expression. There is also some evidence that T₃ has direct actions on mitochondria and exerts a degree of control over oxidative phosphorylation.

(3) Investigation of Thyroid Function

In common with reviews of the investigation of adrenocortical function, most reviews of the investigation of thyroid function concentrate on the

use of tests to confirm or exclude specific thyroid disorders in otherwise healthy individuals. However, in contrast to the investigation of adrenocortical function, most standard tests of thyroid function have been validated in severe illness. The background to the tests used in this thesis will be given below. The results of previous studies in severe illness will be reviewed in Chapter 6.

Circulating Thyroid Hormone Concentrations

Serum T4 is the most useful first line test of thyroid function. It is now measured by radioimmunoassay or by enzyme-mediated immunoassay techniques. Knowledge of the state of thyroid protein binding is required for strictly accurate interpretation of results, but is not usually investigated routinely. The normal adult range of total T4 is 60 -160 nmol/L, with small variations between laboratories. T3 is measured by radioimmunoassay and ranges from 1.2 - 3.4 nmol/L. T4 concentrations remain constant in old age, but T3 levels tend to fall. This is most probably a result of the increasing frequency of illness with age.

No special patient preparation is necessary when taking blood for thyroid hormone concentrations. Hormone levels are unaffected by exertion, posture, or the stress of venepuncture. There is no detectable circadian rhythm of T4, but a minor rhythm in T3 concentration has been documented (Nimalasuriya et al, 1986). The same principals of performance and validation of immunoassays detailed in Chapter 2 apply to assays of thyroid hormones.

The concentrations of the free fractions of thyroid hormones are now used increasingly frequently as an alternative to total hormone levels in screening healthy individuals for thyroid disease. There are, however, considerable difficulties in the interpretation of both free thyroid hormone indices and free hormone concentrations during severe illness (Slag et al, 1981; Kaptein et al, 1981; Becket et al, 1991). No attempt was made to measure free thyroid hormones in this study.

Thyrotropin Concentration

Measurement of serum TSH is used primarily as a screening test for hypothyroidism. The first generation of TSH RIAs were accurate within the mid range of normal TSH values and accurately measured high values. They were not able to differentiate between low normal values and suppressed values found in hyperthyroidism. These assays were not able to

provide quantitative TSH values below 1 mU/L. Approximately 10% of euthyroid individuals had TSH levels which were unmeasurably low.

In recent years, with the advent of ultrasensitive TSH assays, it has become possible to detect very low concentrations of TSH in hyperthyroid individuals (Wehmann et al, 1983; Eggersten et al, 1988). These immunoradiometric assays use 2 or 3 monoclonal antibodies targeted against specific epitopes of the TSH molecule. This produces a more stable radioligand and a higher assay sensitivity. These assays are accurate down to TSH concentrations of 0.1 mU/L. Even greater assay sensitivity is possible with immunochemiluminometric assays, which have working sensitivities down to 0.05 mU/L and can easily separate euthyroid from hyperthyroid patients (Weeks et al, 1984). Confirmation of TSH suppression by TRH testing is therefore less often required.

Despite the increased sensitivity of modern TSH RIAs, the specificity has remained high with little significant cross reactivity with LH, FSH or hCG. The finding of a significant proportion of euthyroid individuals with subnormal TSH levels measured using sensitive assays has led to some diagnostic confusion. Recent studies of the significance of subnormal TSH levels in various clinically euthyroid populations are discussed in Chapter 6.

Thyrotropin Releasing Hormone Stimulation Test

The TRH stimulation test provides a standard supraphysiological challenge to pituitary TSH secretion. It allows the measurement of the intrinsic TSH secretory reserve and the extent to which TSH secretion is inhibited. TRH is effective in activating TSH secretion whether given orally, intramuscularly, or intravenously. The standard test involves the intravenous administration of either 200 or 400 mcg TRH which produces a maximal TSH response. In the UK, 200 mcg TRH is the standard dose (Ismail, 1981), whereas physicians in Europe and the USA tend to use 400 mcg. Irrespective of TRH dosage, the TSH concentration rises rapidly, reaching a peak at 20 - 30 minutes. In normal individuals a TSH increment of between 5 and 30 mU/L is expected, with a mean increment of around 15 mU/L. In premenopausal women there is minor variation in the TSH response with the menstrual cycle, and there is some decline in the TSH increment with age. The factors discussed above, which inhibit or augment basal TSH concentration tend to have the same effect on the TSH increment following TRH.

In clinical practice the TRH test is used most frequently to confirm the diagnosis of hyperthyroidism. A normal TSH response excludes thyrotoxicosis. In primary hypothyroidism the TSH response is accentuated, although, in the presence of an high basal TSH level, such confirmation is not usually necessary. Suspected hypothyroidism with a low or normal TSH concentration suggests pituitary or hypothalamic hypothyroidism. The TRH test is of some use in this context. In pituitary hypothyroidism the TSH response is small or absent, whereas in hypothalamic hypothyroidism the response is normal but delayed. This approach is, however, far from infallible. TRH stimulation often leads to small increments in thyroid hormone concentrations, but the inconsistent response is of no diagnostic value, and is not routinely measured. The use of the TRH test during illness will be discussed in Chapter 6.

There are no recognised contra-indications to TRH testing, although caution is recommended in patients suffering from asthma and myocardial ischaemia. The test is well tolerated, with only mild side effects such as nausea, facial flushing, and a strange taste in the mouth.

Thyrotropin Stimulation Test

The TSH stimulation test involves the administration of purified bovine TSH and the measurement of the thyroid hormone response. The test formerly played a prominent role in the diagnosis of hypothyroidism, but is now rarely used in clinical practice. It is occasionally used to determine whether a patient taking thyroxine therapy has primary hypothyroidism. It can be used to investigate suspected subclinical primary hypothyroidism and to assess thyroid reserve. It may be used to differentiate between primary and secondary hypothyroidism. Stimulation with exogenous TSH in conjunction with thyroid scintiscans is used to assess whether or not thyroid nodules are functional. Used in this manner it is of value in detection of remnants of thyroid carcinoma. Finally, bovine TSH may be used therapeutically as an adjunct in the management of certain types of functioning thyroid carcinoma.

Protocols for the performance of TSH stimulation tests vary depending upon which of the above uses the test is to be put. The investigation of patients receiving thyroxine replacement, the differentiation between primary and secondary hypothyroidism, and the investigation of patients with longstanding myxoedema require daily administration of TSH for 3 days (Querido and Stanbury, 1950; Skanse, 1953). To aid the diagnosis of

thyroid cancer remnants requires administration for up to 7 days. A single dose of TSH may be used to assess borderline or subclinical hypothyroidism and to determine thyroid reserve (Fore and Wynn, 1966)). A single dose as low as 1 IU has been used (Perlmutter et al, 1952), but 5 IU is required to produce a maximal thyroid response (Faber et al, 1976) and 10 IU is the recommended standard dosage (Taunton et al, 1965).

The expected response to the subcutaneous or intramuscular administration of a single 10 IU dose of TSH is well defined. In normal individuals the thyroid radioiodine uptake and plasma protein-bound iodine are increased at 4 hours, peak at 12 to 18 hours and begin to decline by 24 hours. The increment of both these indices range from 25 to 50% in normal individuals and less than 10% in hypothyroid patients (Jefferies et al, 1959). The serum T4 increment should be at least 20 nmol/L (Burke, 1968). The fractional increase in T3 concentration is usually greater than T4 (Faber et al, 1976).

A low thyroid reserve in clinically euthyroid individuals is found after ^{131}I treatment or surgical therapy for Grave's Disease, and also in Hashimoto's thyroiditis (Jefferies et al, 1956). The significance of a low thyroid reserve in clinically euthyroid patients is unclear. In one study 10 such patients, with no history of thyroid disease, were identified (Burke, 1968). On follow-up for a mean of 4 years only 2 of the 10 patients developed clinical hypothyroidism. There has been virtually no work on TSH testing published over the past 20 years, and there have been no studies of TSH testing during illness.

TSH testing is a relatively safe procedure. Exogenous TSH is contraindicated following myocardial infarction, in cardiac failure, and in untreated Addison's disease. There were no serious toxic reactions in the 87 euthyroid patients studied by Taunton et al (1965) or the 211 patients studied by Burke (1968). Pain at the site of injection is not infrequent, and very occasionally urticaria may develop. In rare instances anaphylaxis has been reported. It has been suggested that patients should be given a small subcutaneous test dose of TSH prior to the full test in order to detect any allergic tendency. Systemic symptoms such as nausea, vomiting, fever and tachycardia are unusual after administration of 10 IU TSH, but are frequent at higher doses (Querido and Stanbury, 1950).

(4) Factors Confounding the Interpretation of Thyroid Function Tests During Critical Illness

The changes in thyroid function which take place during illness were detailed in Chapter 1. A number of other factors complicate the interpretation of these changes in an intensive care population.

Age and Thyroid Function

Critical illness increases in frequency with age. The effects of age on thyroid function must, therefore, be considered before interpreting the interrelationships between thyroid hormone concentrations, illness and mortality. Total T4 concentration is normal or only slightly decreased in old age (Hesch et al, 1976; Nishekawa et al, 1981; Kabadi and Rosman, 1988), although metabolic clearance and T4 turnover are decreased (Gregerman et al, 1962). Free T4 concentration remains normal with advancing age (Lipson et al, 1979). Many studies have demonstrated a decline in serum T3 with increasing age (Jefferys et al, 1972; Nishekawa et al, 1981; Wartofsky and Burman, 1982), but this may be due to the high frequency of concomitant illnesses in the elderly population (Olsen et al, 1978; Simons et al, 1990). Basal TSH levels are normal or mildly elevated (Hermann et al, 1974; Lipson et al, 1979) with advancing age, while the TSH response to TRH may be blunted (Snyder and Utiger, 1972; Demeester-Mirkine et al, 1981; Finucane et al, 1991). It has been suggested that a normal TSH may not be a valid index of euthyroidism in the elderly, since only 50% of aged patients will augment their TSH response to TRH after iodine-induced decrements in their serum T3 and T4 levels (Ordene et al, 1981).

Drugs and Thyroid Function

The effects of some drugs on TSH secretion and TBG concentration are discussed above. However, a number of other drugs are worthy of mention. Amiodarone, a heavily iodinated antiarrhythmic agent, is a potent inhibitor of 5'-monodeiodinase for T4. Administration leads to a decline in T3 concentration and an increase in rT3 concentration. Amiodarone is also apt to induce hyperthyroidism or hypothyroidism, probably as a result of iodine release as the drug is metabolised. Propranolol is also capable of inhibiting peripheral 5'-monodeiodinases. Again serum T3 falls and rT3 increases, but TSH concentration and responsiveness to TRH are un-

changed. Small intravenous doses of heparin are followed within a few minutes by increases in the serum T4 value and the percentage of free T4. Serum T3 remains normal but TSH concentration is decreased. Inhibition of T4 protein binding is thought to be involved.

Surgery and Thyroid Function

The serum total and free T3 levels fall rapidly after surgery (Adami et al, 1978; Chernow et al, 1983), and are related to the severity of the surgical trauma and prognosis (Philips et al, 1981). T4 levels fall on the day following surgery and rT3 levels rise (Kehlet et al, 1979). TSH levels measured with sensitive assays fall following surgery (Chu et al, 1991). If recovery is uncomplicated, hormone levels tend to normalise by the 5th postoperative day.

Nutrition and Thyroid Function

In the first few days of severe illness patients are frequently deprived of nutrition. In the absence of illness this would alter thyroid function and is therefore a potential confounding variable in the analysis of the response of the H-P-T axis to illness. With total caloric deprivation the serum T3 concentration falls within 24-48 hours, rT3 levels rise and serum T4 may fall (Burman et al, 1979; O'Brian et al, 1980; Borst et al, 1983). The basal TSH concentration and the TSH response to TRH are usually diminished (Borst et al, 1983). Refeeding with glucose returns hormone concentrations to normal if given orally (Burman et al, 1979), but not if given intravenously (Westgren et al, 1977). Refeeding with fat or protein has little effect (Azizi, 1978; O'Brian et al, 1980).

In calorie-deprived patients, the pulse rate decreases and the QKd interval and systolic time interval are prolonged (Meyers et al, 1980). Basal metabolic rate decreases (Huang et al, 1981), and can be restored to normal with T3 replacement (Mince et al, 1980). On the basis of this evidence it has been suggested that starvation leads to an hypothyroid state intended to limit catabolism (Wartofsky and Burman, 1982). Whilst, not proven, it would be surprising if evolution had not led to the development of such a response.

Renal Failure and Thyroid Function

When considering the effect of acute renal failure on thyroid function,

it is difficult to distinguish between effects of renal failure and effects of coexisting illness. However, the changes in thyroid hormone levels during severe illness are reported to be similar in patients with and without acute renal failure (Kaptein et al, 1981). The main difference is a smaller increase in rT3 levels in patients with renal failure. The same problem arises in patients with chronic renal failure, but certain changes do appear to be associated with chronic renal failure, irrespective of associated illness. Serum T4 and free T4 are reduced to subnormal levels in approximately 25% of cases and TSH levels tend to be low normal (Hardy et al, 1988; Van Leusen and Meinders, 1982). Following haemodialysis T3 and TSH remain normal, but T4 and free T4 rise, returning to normal after 48 hours (Van Leusen and Meinders, 1982). Haemodialysis should be avoided during dynamic tests of thyroid function, but acute renal failure *per se* is unlikely to be a significant confounding factor.

Chapter 6

A Study of Thyroid Function During Critical Illness

(1) Aims

(2) Patients and Methods

- (a) Measurement of Thyroid Hormones and TSH on Admission
- (b) Estimation of Metabolic Rate
- (c) Thyrotropin Releasing Hormone Tests
- (d) Thyrotropin Stimulation Tests

(3) Results

(4) Figures

(5) Discussion

(6) Conclusion

(1) Aims

The main aims of the studies detailed in this chapter were as follows:

- 1) To determine the range of concentration of TSH during critical illness using a sensitive assay in patients not receiving dopamine or steroids.
- 2) To determine the range of concentrations of total thyroxine and total triiodothyronine and to relate the concentration of these hormones to metabolic rate, severity of illness and mortality.
- 3) To determine the range TSH responses to a standard TRH

test during critical illness using a sensitive assay in patients not receiving dopamine or steroids.

4) To determine the thyroid hormone response to a TSH stimulation test during severe illness.

(2) Patients and Methods

The background to the tests used in this chapter was discussed in Chapter 5 and details of the patients investigated and the setting of the study were given in Chapter 2.

(a) Measurement of Thyroid Hormones and TSH on Admission

Blood was taken for measurement of serum TSH, thyroxine, and triiodothyronine in 260 consecutive admissions not receiving steroids or dopamine. If possible, blood was taken prior to commencement of sedatives or inotropes. A note was taken of all medications taken in the past 24 hours. APACHE II scores were calculated on data obtained during the first 24 hours of admission. The physicians caring for the patients in the study had access to the thyroid function results. Plasma cortisol concentration was measured on blood sampled at the same time as the blood for measurement of thyroid function. In patients in whom TSH was raised on admission, a repeat blood sample was taken following recovery and discharge from the ICU. No set time after discharge was specified.

(b) Estimation of Metabolic Rate

Metabolic rate was estimated indirectly by measuring carbon dioxide production. Carbon dioxide production was measured

in 100 consecutive patients who were ventilated on admission to the ICU or within the first 12 hours of admission. A measurement was made within 12 hours of admission or ventilation and repeated 12 hours later.

All expired respiratory gases were collected from the ventilator "exhaust port" using a 150 litre Douglas bag. Between 100 and 150 litres of gas was collected over an accurately timed period. A sample of the gases was obtained, using a glass syringe, and passed through an automatic blood gas analyser [Investigation Laboratories 1312]. Readings of the partial pressure of carbon dioxide, the ambient atmospheric pressure and the temperature of the sample were obtained. The volume of gas was measured by passing the contents of the Douglas bag through a modified gas meter, giving a reading of volume in cubic feet which was then converted to litres. Respiratory gases were collected with the patient at rest and procedures such as physiotherapy and haemodialysis were avoided.

All the above data were then entered into a computer containing a software package which calculated the metabolic rate of the patient using the rate of carbon dioxide production and assuming a respiratory quotient of 0.8. A measurement of the weight of the patient, obtained on the day of admission to the ICU, was used to correct the metabolic rate for body mass.

(c) Thyrotropin Releasing Hormone Tests

Thyrotropin releasing hormone stimulation tests were per-

formed on 30 patients and 8 healthy controls. Patients investigated fulfilled the following criteria: a) acute illness of less than 28 days duration; b) no history of thyroid or pituitary disease; c) no dopamine or steroid medication for at least 72 hours; d) APACHE II score greater than 15. Patients were investigated at any point in their ICU stay, and no time of day was specified.

Blood was sampled for measurement of basal TSH concentration. An intravenous injection of 400 mcg TRH (TRH-Roche, Roche Products Limited, Hertfordshire, UK) was then administered and further blood samples taken 20 and 60 minutes after the injection. Blood transfusion and haemodialysis were avoided during the tests, but no other limitations were specified. Measurements of thyroxine and cortisol were made on blood taken at the time of the basal TSH sample. APACHE II scores were calculated on data obtained on the day of the test.

(d) Thyrotropin Stimulation Tests

Thyrotropin stimulation tests were performed in two groups of patients. All patients had an acute illness of less than 28 days duration, no past history of thyroid or pituitary disease, no steroid or dopamine medication for the past 72 hours and an APACHE II score of greater than 15. Patients in Group 1 had T4 concentration less than 20 nmol/L, TSH concentration less than 0.5 mU/L and a TSH increment following TRH of less than 5 mU/L. Patients in Group 2 had a T4 concentration of greater than 60 nmol/L and TSH concentra-

tion was greater than 2.0 mU/L. Group 1 comprised 7 patients and Group 2 comprised 6 patients.

Twelve hours before the full TSH stimulation test was performed, a small subcutaneous injection of TSH was given. This comprised 0.25 units of bovine TSH in 0.5 ml saline. If there was any evidence of a local or systemic allergic reaction over the subsequent 12 hours the full TSH test was not performed.

Blood was sampled for a basal measurement of TSH, thyroxine, and triiodothyronine concentrations. Immediately after the basal sample was taken an intramuscular injection of 10 units of bovine TSH (Thyrotropar, Armour Pharmaceutical company, Eastbourne, Sussex, UK) was given. The TSH was diluted with 5 ml 0.9% saline prior to injection. All injections were given into deltoid muscle. Blood was taken for measurement of total T4 and T3 concentrations at 4, 12 and 24 hours after injection. No limitations were placed on the timing of the test or on patient management during the test, other than that no haemodialysis or blood transfusion should take place.

(3) Results

Thyrotropin Concentration

Measurements of TSH, thyroxine, triiodothyronine and cortisol were available in 260 cases. The distribution of thyrotropin concentration was non-parametric with a tail of higher values [Fig 6.1]. Logarithmic transformation gave a normal distribution [Fig 6.2]. Seventy four patients [29%] had TSH concentrations outside the normal range of the assay used [0.4 - 5.0 mU/L]. Thirty nine patients had low TSH concentrations, of whom 15 had concentrations below 0.1 mU/L. Thirty five patients had high TSH concentrations, none of whom had a past history of thyroid disease. Mean [SD] admission TSH concentration was 2.58 [3.35] mU/L. The median TSH concentration was 1.6 mU/L [range < 0.1 - 21.0].

There was no relation between time of admission, age or sex and TSH concentration. There was a negative correlation between TSH concentration and APACHE II score [$r = -0.355$, $P < 0.01$].

Table 6.1. TSH concentrations on admission in 260 patients according to APACHE II score.

APACHE SCORE	< 16	16-24	> 24
NUMBER OF CASES	89	105	66
TSH [mU/L]:			
MEAN [95% CI]	3.6 [2.8-4.4]	2.3 [1.9-2.7]	1.4 [0.9-1.8]
MEDIAN [RANGE]	2.4 [<0.1-21]	1.6 [<0.1-13]	1.0 [<0.1-8]
95% RANGE	0.4 - 16	0.1 - 10	< 0.1 - 5

The mean, median and 95% range (obtained by log transformation) of TSH concentration is given within the three standard APACHE II score groups in Table 6.1. The mean APACHE II scores differed significantly between the three groups. Each of the groups contained TSH concentrations above and below the normal range. However, TSH concentrations below the normal range for health [0.4 mU/L] are outwith the 95% range for patients with APACHE II scores <16, and concentrations above the normal range for health [5.0 mU/L] are outwith the 95% range for patients with APACHE II scores > 24. Mortality decreased as TSH concentration increased [Fig 6.3]. Mortality of patients with TSH concentrations below the normal range in health was 72%(95% CI, 55-85) compared with 30% (95% CI, 24-37) among patients with TSH concentrations within the normal range and 11% (95% CI, 2-27) in patients with concentrations above the normal range (χ^2 for trend, $P<0.001$, Table 6.2).

Table 6.2. Mortality, thyroxine [T4], triiodothyronine [T3], cortisol and APACHE II scores in patients with low, normal and high TSH concentrations [mean (SD)]

TSH mU/L	CASES	DEATHS [%]	T4 nmol/L	T3 nmol/L	CORTISOL nmol/L	APACHE II SCORE
<0.4	39	28 [72]	26 [13]	0.21 [0.1]	1692 [741]	32 [11]
0.4-5	186	56 [30]	69 [21]	0.34 [0.16]	974 [470]	22 [10]
>5.0	35	4 [11]	83 [23]	0.62 [0.19]	587 [274]	16 [7]

TSH concentration correlated positively with T4 concentration [$r = 0.48$, $p < 0.001$, Fig 6.4] and negatively with cortisol concentration [$r = -0.57$, $p < 0.001$, Fig 6.5]. The correlation between TSH concentration and T3 was less marked, but remained significant [$r = 0.26$, $p < 0.05$].

TSH concentrations were higher in patients who subsequently survived than in those who died [Table 6.3]

Table 6.3. Median [range] concentrations of TSH and cortisol, and mean [SD] age and APACHE II scores in survivors and nonsurvivors.

	SURVIVORS	NONSURVIVORS	TOTAL
CASES	172	88	260
AGE [yrs]	57.2 [16]	60.7 [15]	58.4 [16]
TSH [mU/L]	2.1 [<0.1-21]	0.6 [<0.1-7.2]	1.6 [<0.1-21]
CORTISOL	716 [162-2950]	1260 [180-3750]	916 [162-3750]
APACHE II SCORE	17.0 [8]	26.5 [9]	20.2 [9.6]

In 21 cases with a raised TSH concentration on admission, a repeat measurement was made after ICU discharge. The mean [SD] TSH on admission was 9.6 [6.1] mU/L, and mean [SD] T4 concentration was 76 [24] nmol/L. Following discharge TSH was within the normal range in 14 cases, and had fallen in 17 cases. The mean [SD] TSH and T4 concentrations after discharge were 6.1 [3.9] mU/L and 89 [28] nmol/L respectively.

Thyroid Hormone Concentrations

The distribution of T4 concentration was approximately parametric [Fig 6.6]. Mean admission T4 concentration was 66 nmol/L [SD = 28]. Thyroxine concentration was below the normal range for the assay [60 nmol/L] in 138 cases. In 14 cases, T4 concentration was below 10 nmol/L. A raised T4 concentration [> 160 nmol/L] was found in 2 cases.

Thyroxine concentration correlated inversely with APACHE II scores [$r = -0.44$, $p < 0.005$] and plasma cortisol concentration [$r = -0.39$, $p < 0.01$]. There was no correlation with age or sex. Admission T4 concentration was related to outcome with mortality increasing as T4 concentration fell [Fig 6.7]. Thyroxine concentration was significantly higher in survivors than nonsurvivors [Table 6.4].

Table 6.4. Median [range] concentrations of thyroxine, triiodothyronine [T3], and cortisol, and mean [SD] age and APACHE II scores in survivors and nonsurvivors.

	SURVIVORS	NONSURVIVORS	TOTAL
CASES	172	88	260
AGE [yrs]	57.2 [16]	60.7 [15]	58.4 [16]
THYROXINE	74 [<10 -177]	37 [<10 -127]	59 [<10 -177]
T3	0.56 [<0.4 -2.6]	<0.4 [<0.4 -1.4]	<0.4 [<0.4 -2.6]
CORTISOL	716 [162-2950]	1260 [180-3750]	916 [162-3750]
APACHE II SCORE	17.0 [8]	26.5 [9]	20.2 [9.6]

Allocation of patients into the three APACHE II score groups produced significantly different mean T4 concentrations (Table 6.5). The 95% range of thyroxine concentration also differed between the three APACHE II score groups. In patients with an APACHE II score < 16, thyroxine concentrations will usually be greater than 40 nmol/L whereas patients with APACHE II scores > 24 will often have thyroxine concentrations of less than 10 nmol/L.

Table 6.5. Mean, median and ranges of thyroxine concentration in patients grouped according to APACHE II score.

APACHE SCORE	< 16	16-24	> 24
NUMBER OF CASES	89	105	66
THYROXINE [nmol/L]:			
MEAN [SD]	82 [19]	55 [31]	33 [29]
MEDIAN [RANGE]	75 [26 - 177]	52 [12 - 146]	30 [<10 - 117]
95% RANGE	40 - 160	20 - 140	<10 - 110

Although nonsurvivors had lower thyroxine concentrations than survivors, mortality was not strongly related to thyroxine concentration within the three APACHE II score groups [Table 6.6]. Overall, mortality fell as thyroxine concentration increased, but this was less evident within the APACHE II score groups. Patients with low thyroxine concentrations did not have a significantly higher mortality than the remainder of their APACHE II score group.

Triiodothyronine concentration was below the normal range of

the assay [1.1 nmol/L] in 182 cases [70%], and below 0.4 nmol/L in 151 cases [58%]. The distribution was non-parametric [Fig 6.8]. Triiodothyronine concentrations were lower in nonsurvivors than survivors [Table 6.4].

Table 6.6. Mortality according to APACHE II score group and admission thyroxine concentration.

APACHE II		% MORTALITY [DEATHS/CASES]			
SCORE:	<16	16-24	>24	TOTAL	
<hr/>					
T4 [nmol/L]					
< 10	0 [0/0]	60 [3/5]	67 [6/9]	64	[9/14]
10 - 30	22 [2/9]	39 [9/23]	71 [15/21]	49	[26/53]
30 - 60	21 [5/24]	29 [8/28]	74 [14/19]	38	[27/71]
> 60	7 [4/56]	31 [15/49]	41 [7/17]	21	[26/122]
<hr/>					
TOTAL	12 [11/89]	33 [35/105]	64 [42/66]	34	[88/260]
<hr/>					

Thyroid Hormones and Metabolic Rate

The mean [SD] APACHE II score of patients studied was 25.2 [9.4]. Thirty eight patients [38%] died. The results detailed below refer to the mean of the two measurements of metabolic rate obtained on each patient. The difference between the two measurements was expressed as a percentage of the mean of the measurements. The mean [SD] variation between the two measurements in the 100 cases studied was 6.8% [2.2].

The mean metabolic rate was 132 kJ/kg/24 hours. There was no

difference between males and females [134 vs 129 kJ/kg/24h]. The predicted basal metabolic rate [PBMR] during health, corrected for weight and sex, was obtained in each case from standard tables. The difference between the measured metabolic rate and the PBMR was expressed as the percentage excess of the former over the latter. This is termed the "excess metabolic rate [EMR]".

In 3 cases, one of whom was hypothermic, the EMR was equal or less than zero. In the remaining 97, cases the EMR was greater than the zero. In 81 cases, the EMR was greater than 25% and in 28 cases it was greater than 50% [Fig 6.9]. Nonsurvivors had a higher mean (SD) EMR than survivors [52% (23) vs 37% (21), MD = 15%, 95% CI = 6.2-23.8]. There was no relationship between EMR and age, sex, or previous surgery. The EMR correlated positively with APACHE II scores [$r = 0.27$, $p < 0.05$] and negatively with admission thyroxine concentration [$r = -0.35$, $p < 0.05$, Fig 6.10]. The EMR also correlated with body temperature [$r = 0.31$, $p < 0.05$]. There was no significant correlation with thyrotropin, triiodothyronine or cortisol concentrations.

TRH Tests

The mean [SD] APACHE II score of patients studied [14 female, 16 male] was 23.4 [5.1]. The mean TSH concentration increased significantly following administration of TRH in both cases and controls [Table 6.7]. The TSH increment was defined as the difference between the basal TSH concentration and the higher of the 20 and 60 minute levels. The

range of TSH increments was greater in cases than controls. In 5 cases there was no detectable TSH increment, and in a further 8 cases the increment was less than 2 mU/L [Figs 6.11 and 6.12]. The mean [SD] TSH increment in the control cases was 11.7 [2.1]. All of the control cases had a TSH increment of greater than 9 mU/L [Fig 6.13]. In 26 [87%] of the ICU cases, the TSH increment was less than 9 mU/L. The median TSH increment in the ICU cases was 3.2 mU/l [range 0 - 14.1]

There was a significant correlation between the basal TSH concentration and the TSH increment following TRH [$r = 0.68$, $p < 0.01$]. The TSH increment correlated positively with T4 concentration [$r = 0.49$, $p < 0.05$] and negatively with cortisol concentration [$r = -0.52$, $P < 0.05$] and APACHE II score [$r = -0.46$, $p < 0.05$]. The mean (SD) TSH increment following TRH was 8.6 [3.1] mU/L in survivors [$n = 19$] and 1.8 [1.3] mU/L in nonsurvivors [MD = 6.8 mU/L, 95% CI = 4.8 - 8.8]. There was no correlation between the TSH increment and either age or sex.

Table 6.7. The mean [SD] thyrotropin response to TRH stimulation tests in cases and controls.

TIME [mins]	THYROTROPIN CONCENTRATION [mU/L]		
	0	20	60
CASES [N=30]	1.05 [0.54]	4.1 [3.4]	5.3 [4.7]
CONTROLS [N=8]	1.15 [0.44]	10.1 [1.3]	14.4 [1.2]

TSH Tests

The mean [SD] APACHE II scores of patients in Group 1 [4 male, 3 female] was 31.1 [4.0], and the mean [SD] score in Group 2 [4 female, 2 male] was 19.1 [2.7]. There were no allergic reactions to the subcutaneous test dose of TSH, and no reactions to the full TSH dose during the test. In the 4 control cases, both T4 and T3 concentrations were increased at 4 hours after injection of TSH, and remained higher at 24 hours [Fig 6.14]. The mean concentration of T4 was significantly higher than the basal level at 12 hours [MD = 34 nmol/L, 95% CI 11.5 - 56.5], but this no longer reached significance at 24 hours [Table 6.8]. The increment in hormone concentration was defined as the difference between the basal concentration and the highest of the subsequent measurements. The mean [SD] increment in T4 concentration in control cases was 33.8 [12.9] nmol/L. The mean [SD] increment in T3 concentration was 1.78 [0.66] nmol/L.

In Group One, concentrations of both T4 and T3 were increased in all 7 cases at 4 hours, and remained higher than the basal level at 24 hours [Fig 6.15]. The mean T4 concentration was significantly higher than the basal level at 4 hours [MD = 37 nmol/L, 95% CI = 17 - 57], and remained so at 12 and 24 hours. The mean T3 concentration was higher than basal at 4 hours [MD = 1.44 nmol/L, 95% CI = 0.74 - 2.14], and remained so at 12 and 24 hours. The mean [SD] increments in T4 and T3 concentration were 48.9 [19.5] and 1.9 [1.3] nmol/L.

In Group Two, concentrations of T4 and T3 were increased in

all 6 cases at 4 hours, and remained higher than their basal concentrations at 12 and 24 hours [Fig 6.16]. The mean T4 level was higher than the mean basal concentration at 4 hours [MD = 18.4 nmol/L, 95% CI = 4 - 23] and 12 hours [MD = 18.2 nmol/L, 95% CI = 8 - 28], but fell back towards the basal level at 24 hour. The mean T3 concentration was higher than basal at 4 hours [MD = 1.21 nmol/L, 95% CI = 0.55 - 1.87] and remained significantly higher at 12 and 24 hours. The mean [SD] increment in T4 concentration was 21.5 [9.3] nmol/L. The mean [SD] increment in T3 concentration was 1.53 [0.82] nmol/L.

Table 6.8. The mean [SD] concentrations of thyroxine and triiodothyronine during thyrotropin stimulation testing.

TIME [hours]	0	4	12	24
CONTROLS:				
T4	100 [14.2]	119 [11.7]	134 [11.7]	120 [12.4]
T3	1.5 [0.41]	2.6 [0.62]	3.3 [0.65]	2.6 [0.58]
GROUP 1:				
T4	13 [3.7]	50 [24.5]	56 [16.3]	49 [11]
T3	0.4 [0.07]	1.9 [0.85]	2.3 [1.28]	1.5 [0.85]
GROUP 2:				
T4	72 [8.3]	91 [13.8]	91 [6.8]	78 [12.9]
T3	0.9 [0.41]	2.1 [0.60]	2.2 [0.87]	1.5 [0.39]

The mean T4 increment in Group One was significantly higher than that in Group Two [MD = 27.4, 95% CI = 8.2 - 46.6]. The mean increment in T3 concentration in Group One was greater than in Group Two, but this did not reach statistical sig-

nificance. The proportional increase in the concentration of both hormones was clearly much greater in Group One than in Group Two. There was a significant fall in the T4 concentration in Group Two between 12 and 24 hours [MD = 13.2 nmol/L, 95% CI = 1 - 26.5], but only a small fall in Group One.

FIG 6.1. THE DISTRIBUTION OF ADMISSION THYROTROPIN CONCENTRATION IN 260 CASES

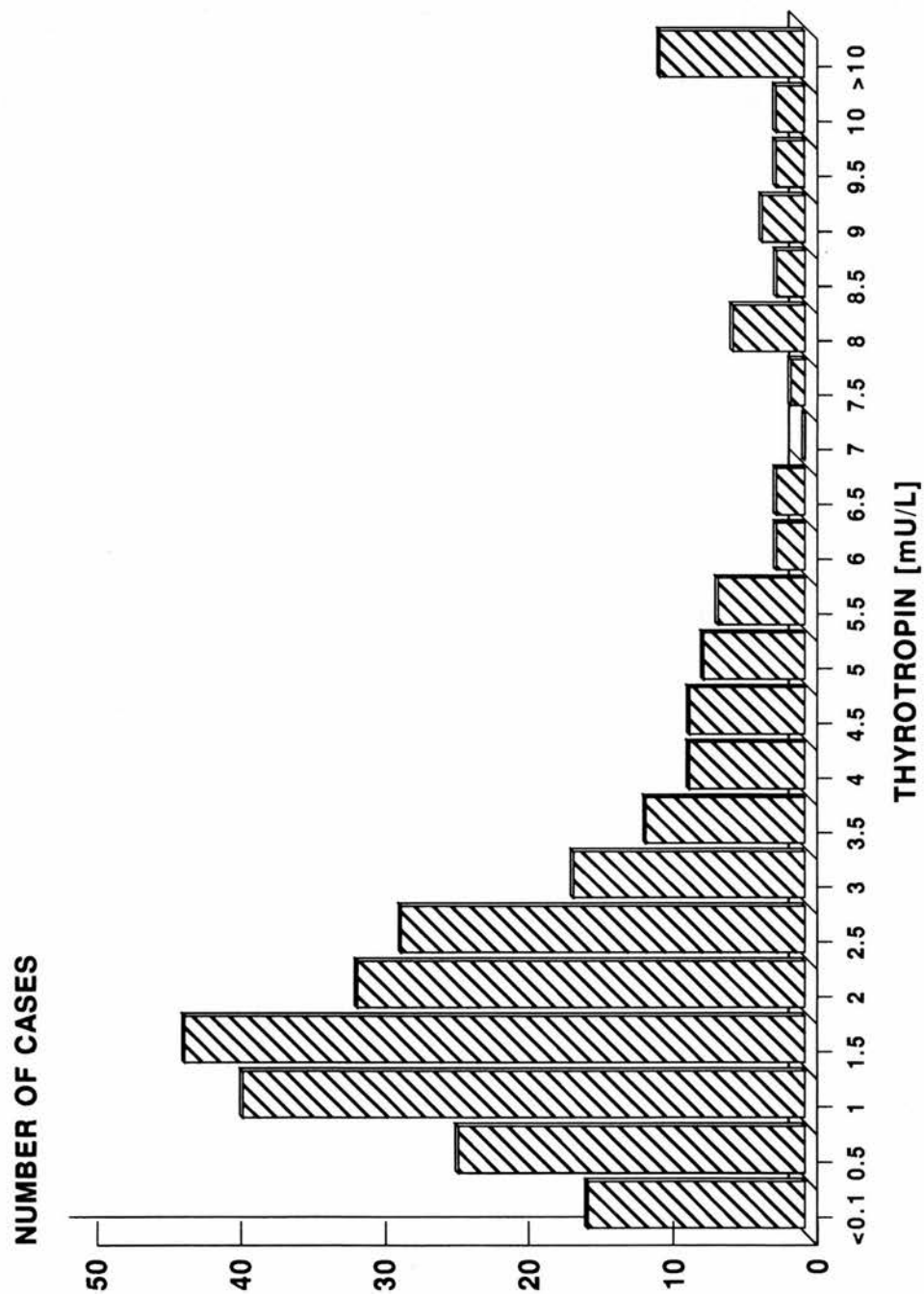


FIG 6.2. THE DISTRIBUTION OF ADMISSION THYROTROPIN
CONCENTRATION IN 260 CASES
LOGARITHMIC SCALE

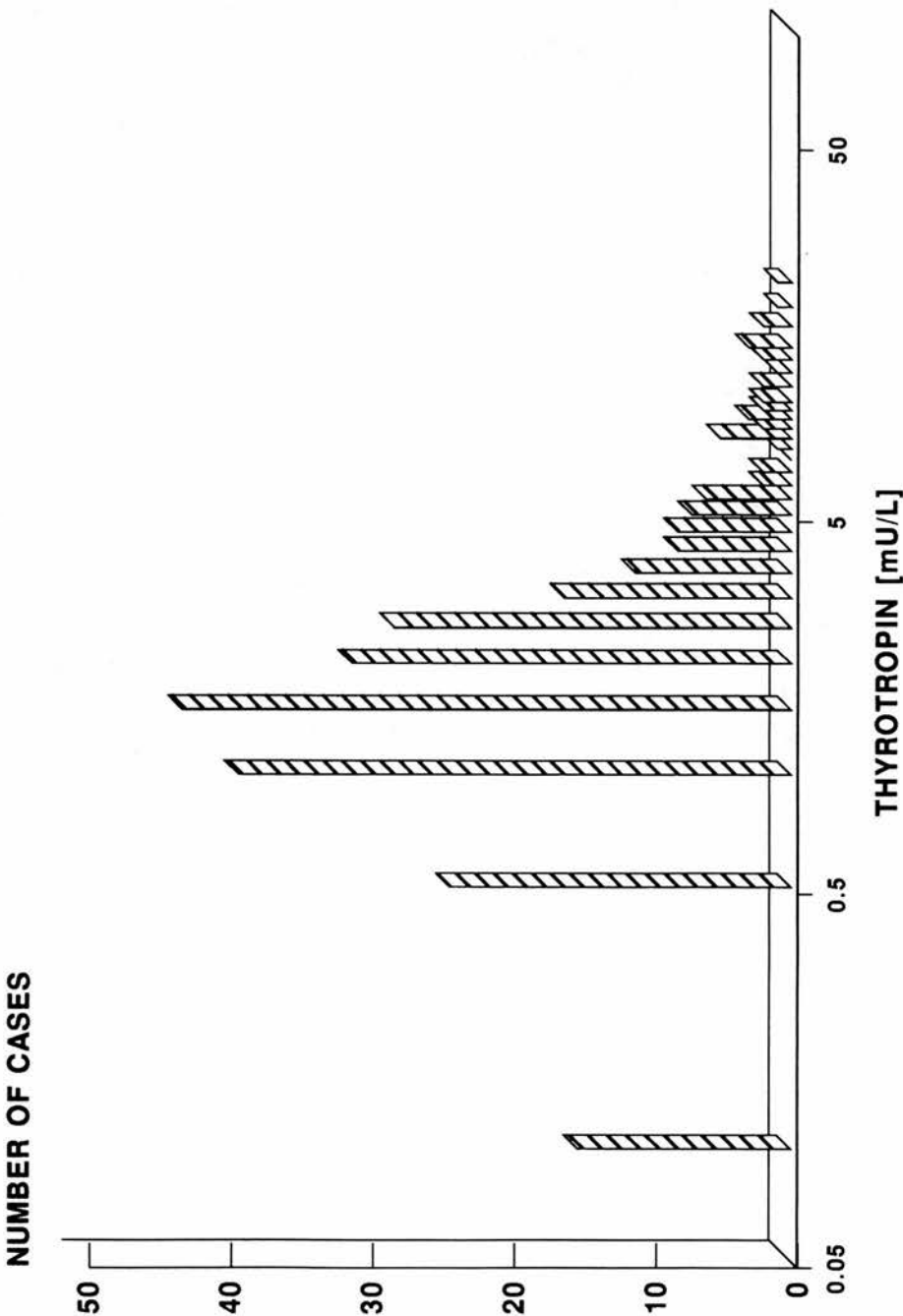
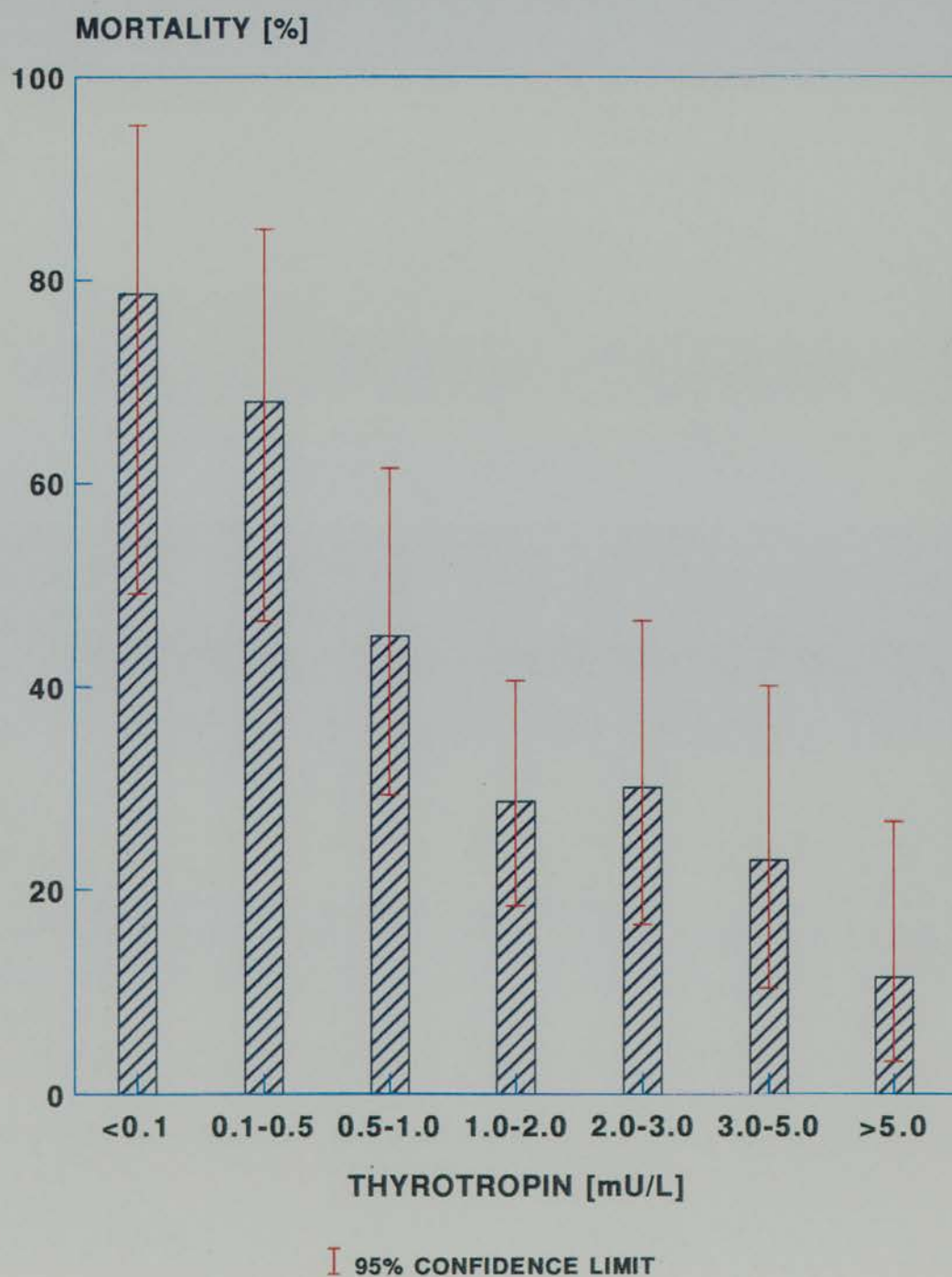


FIG 6.3. MORTALITY IN PATIENTS GROUPED ACCORDING TO ADMISSION THYROTROPIN CONCENTRATION



**FIG 6.4. ADMISSION THYROTROPIN CONCENTRATION VERSUS
THYROXINE CONCENTRATION IN 260 CASES**

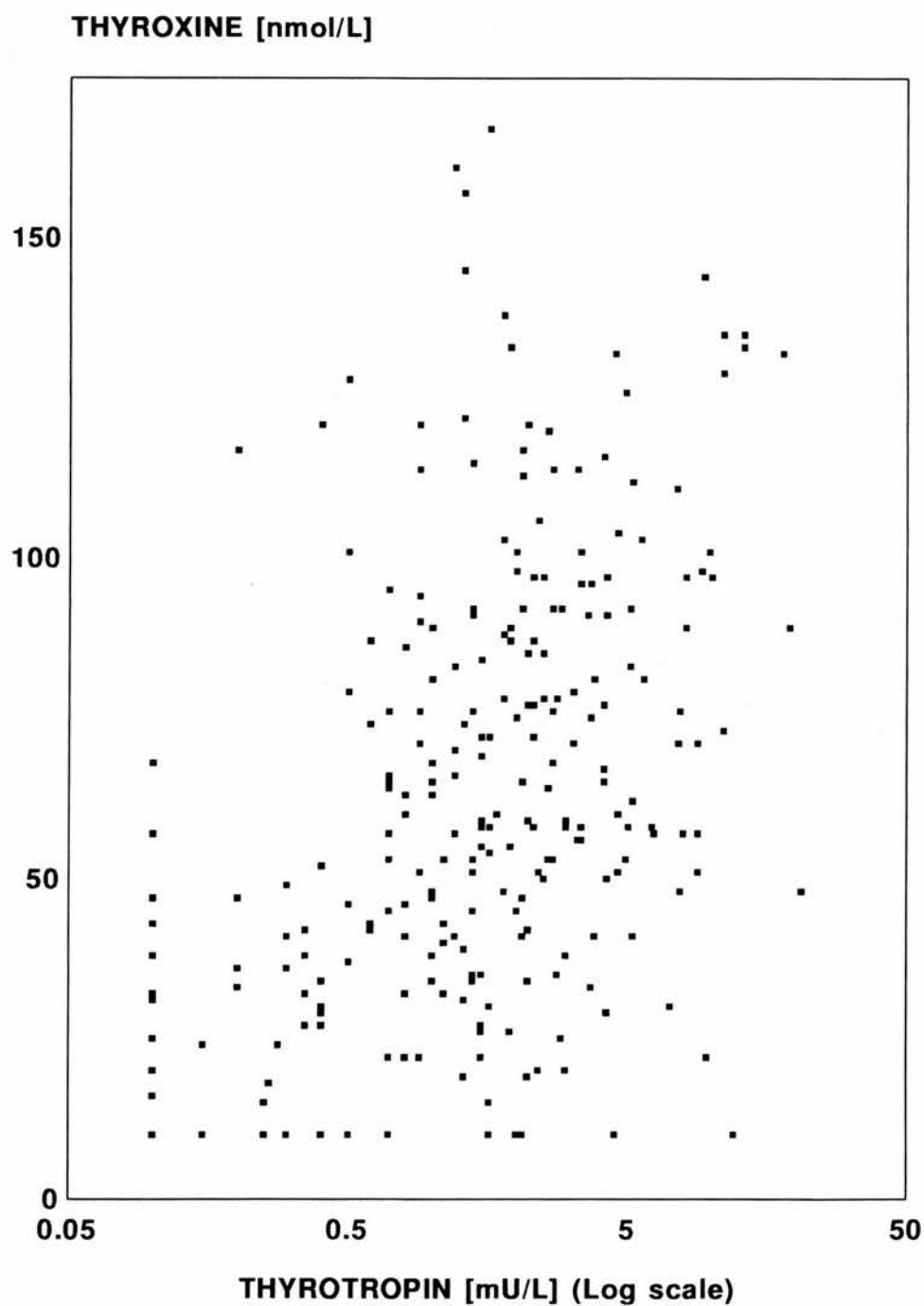


FIG 6.5. ADMISSION THYROTROPIN CONCENTRATION VERSUS PLASMA CORTISOL CONCENTRATION IN 260 CASES

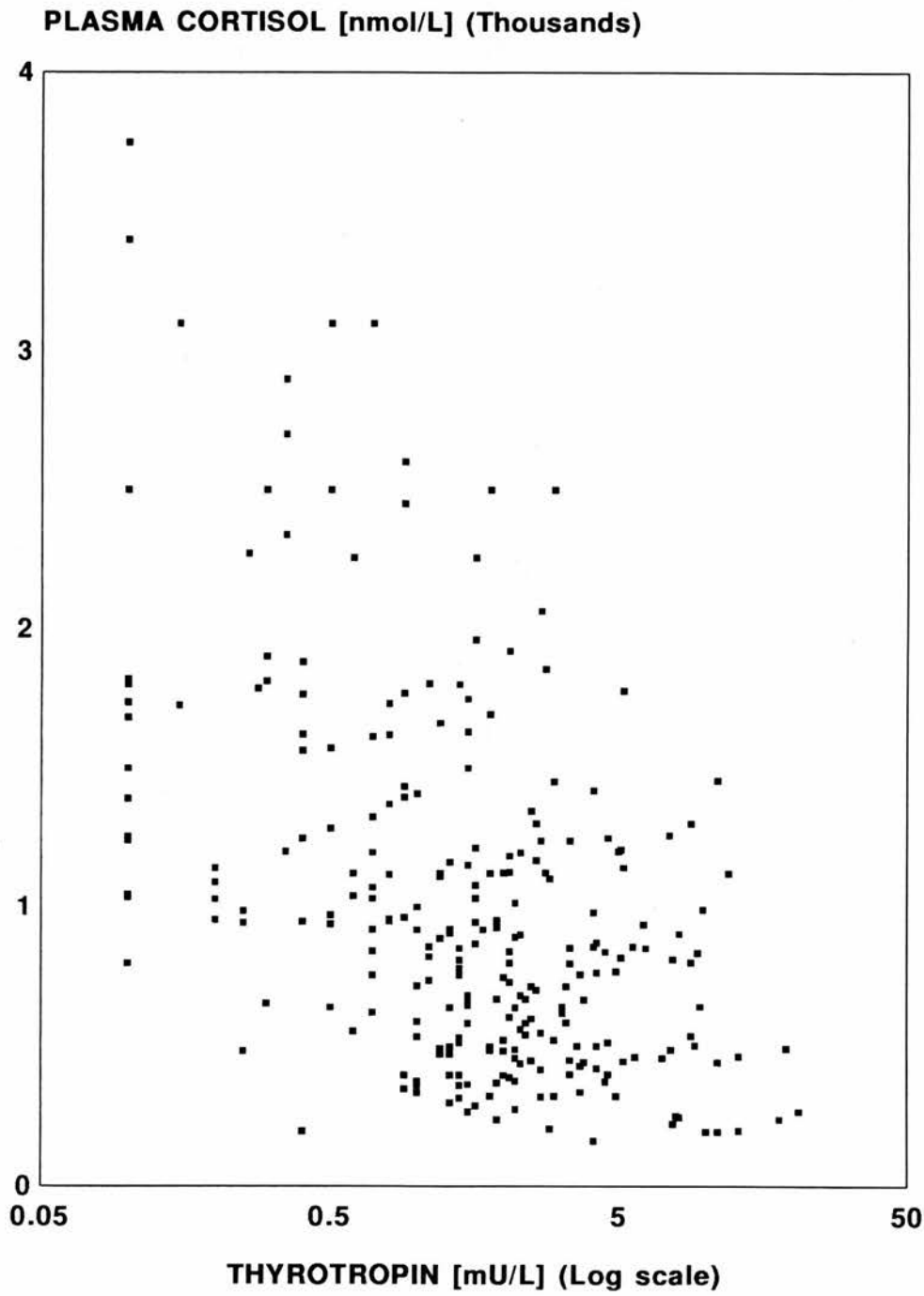


FIG 6.6. THE DISTRIBUTION OF ADMISSION THYROXINE
CONCENTRATION IN 260 CASES

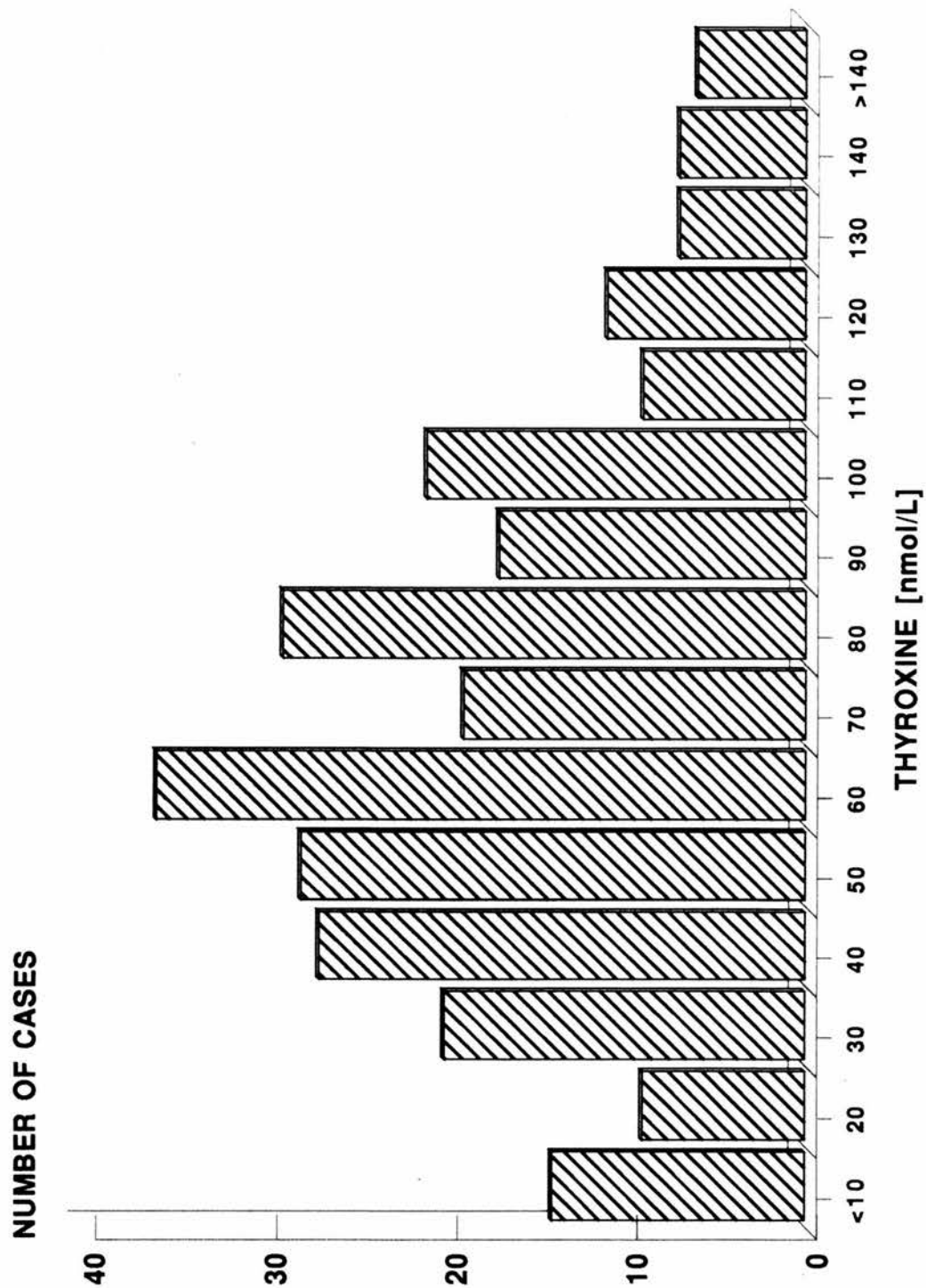
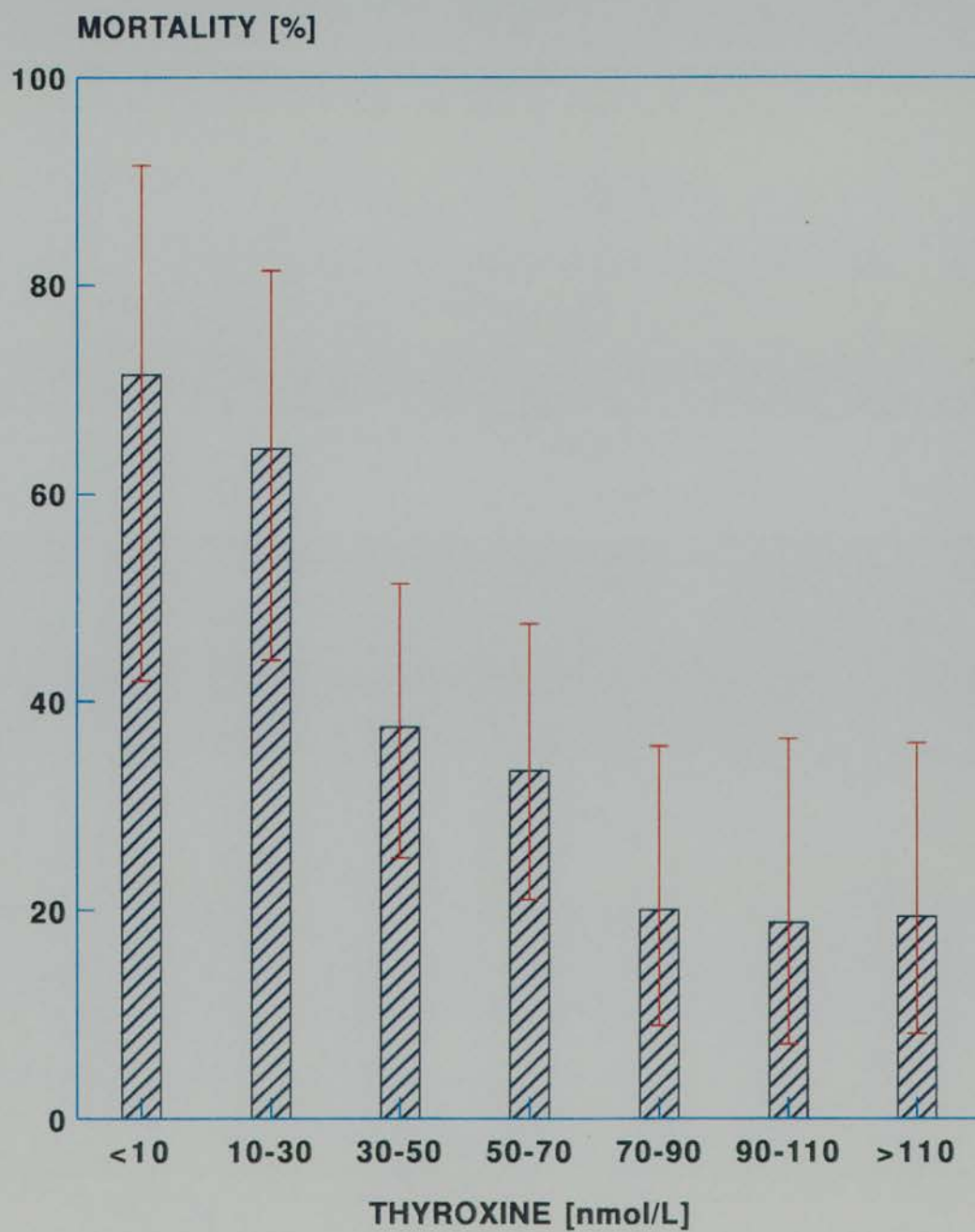
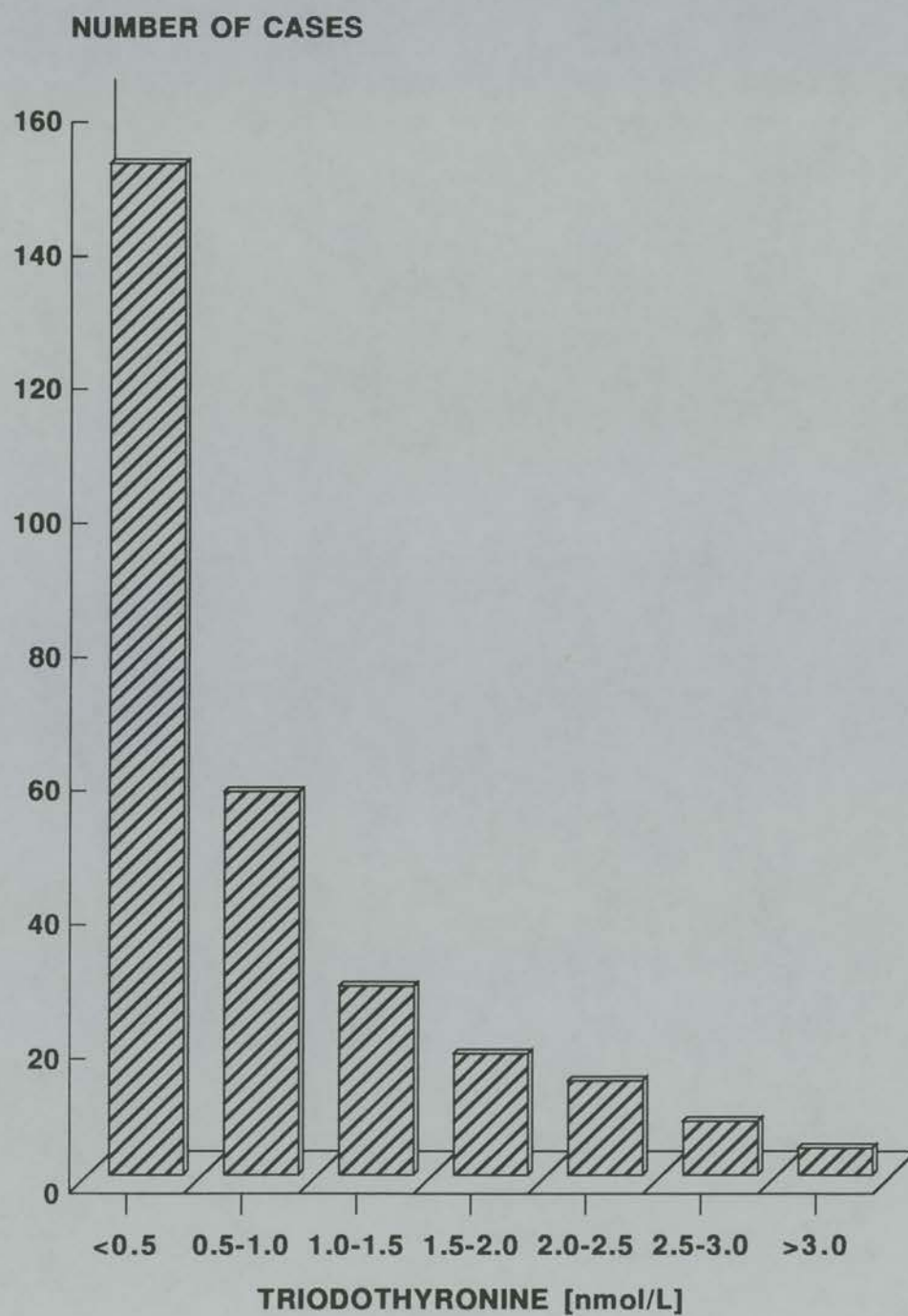


FIG 6.7. MORTALITY IN PATIENTS GROUPED ACCORDING TO ADMISSION THYROXINE CONCENTRATION

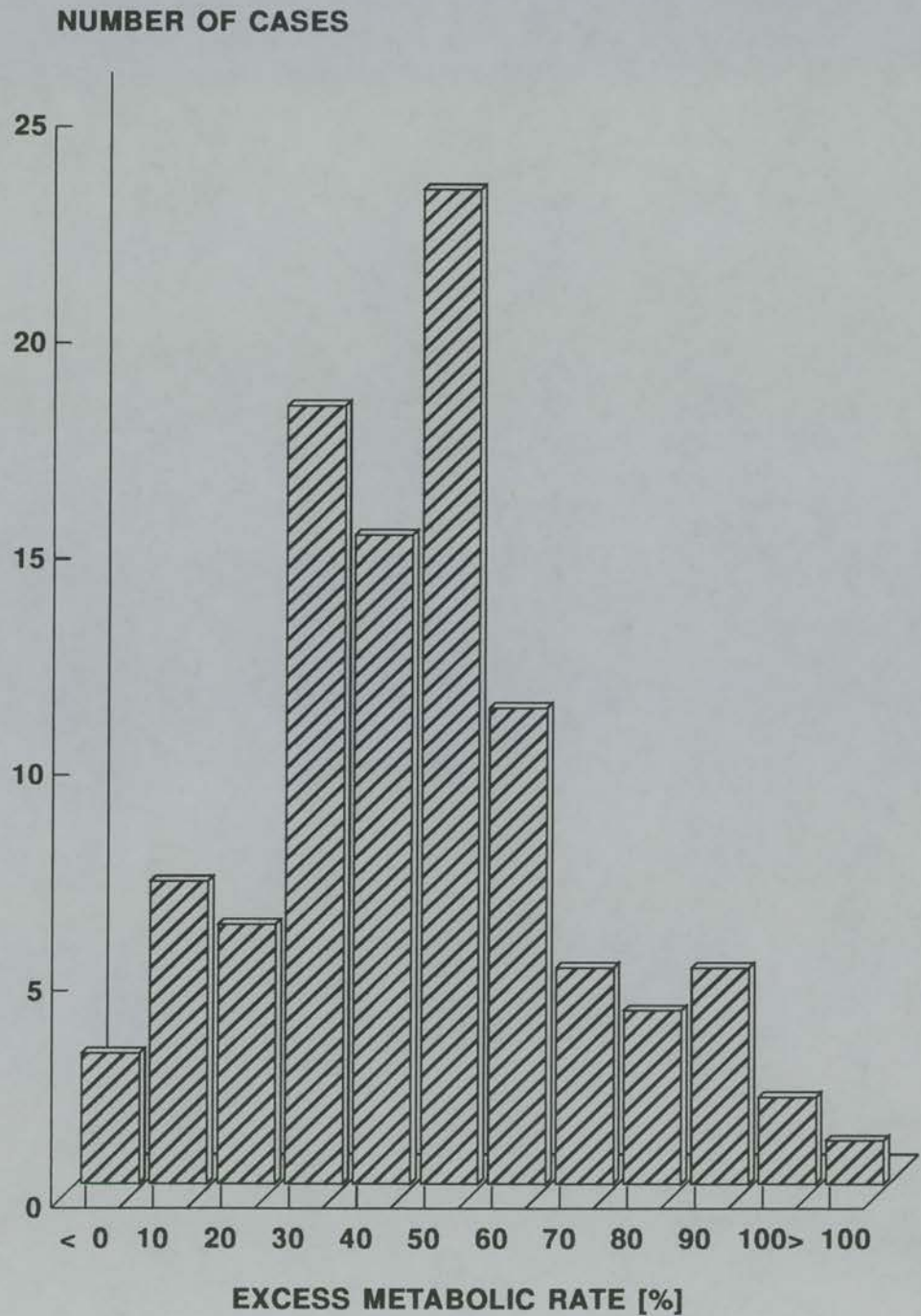


I 95% CONFIDENCE LIMIT

FIG 6.8. THE DISTRIBUTION OF ADMISSION TRIIODOTHYRONINE CONCENTRATION IN 260 CASES



**FIG 6.9. THE DISTRIBUTION OF EXCESS METABOLIC RATE
IN 100 CASES**



**FIG 6.10. THYROXINE CONCENTRATION ON ADMISSION VERSUS
EXCESS METABOLIC RATE**

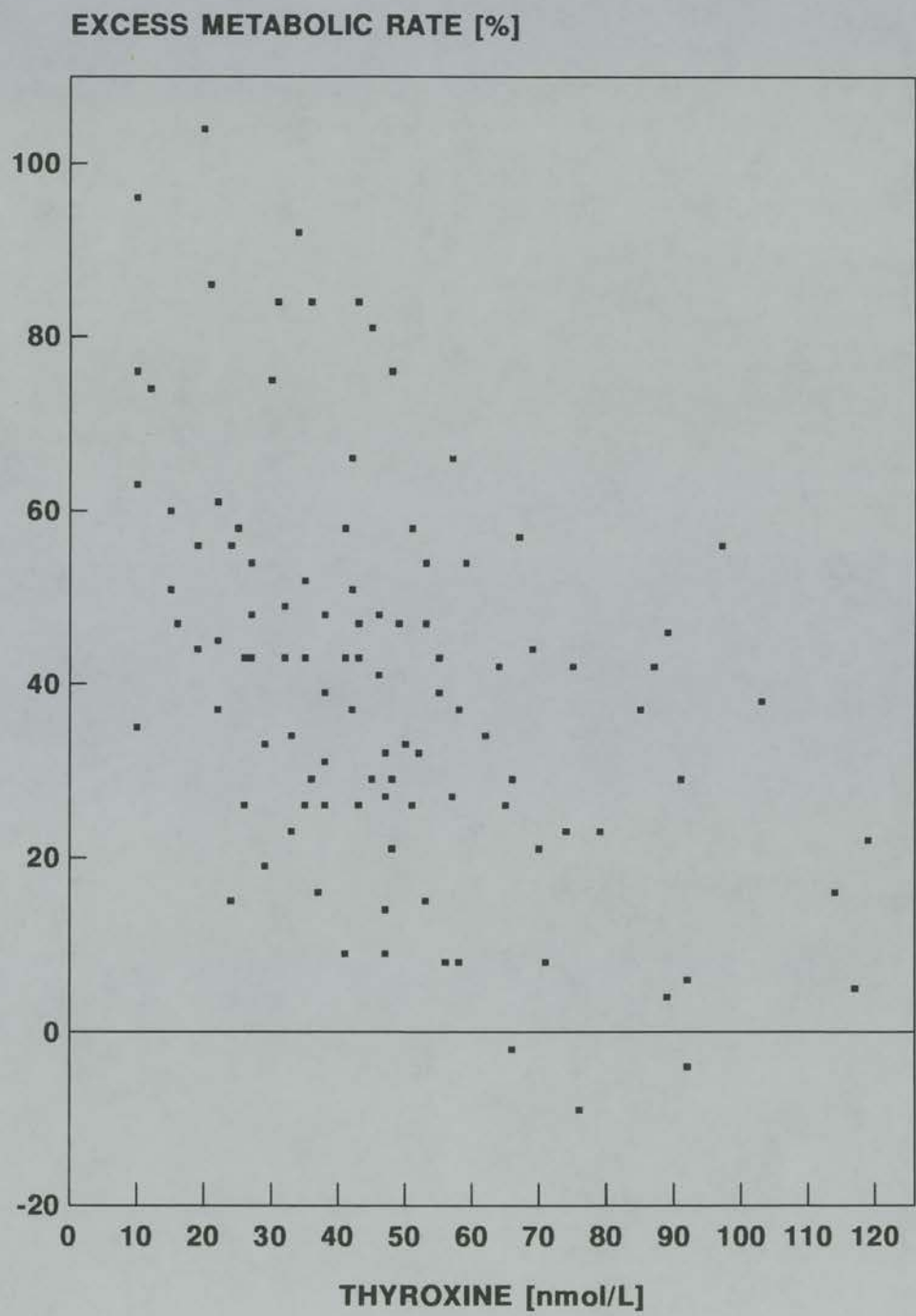


FIG 6.11. THYROTROPIN RESPONSE TO TRH TESTING IN ICU CASES

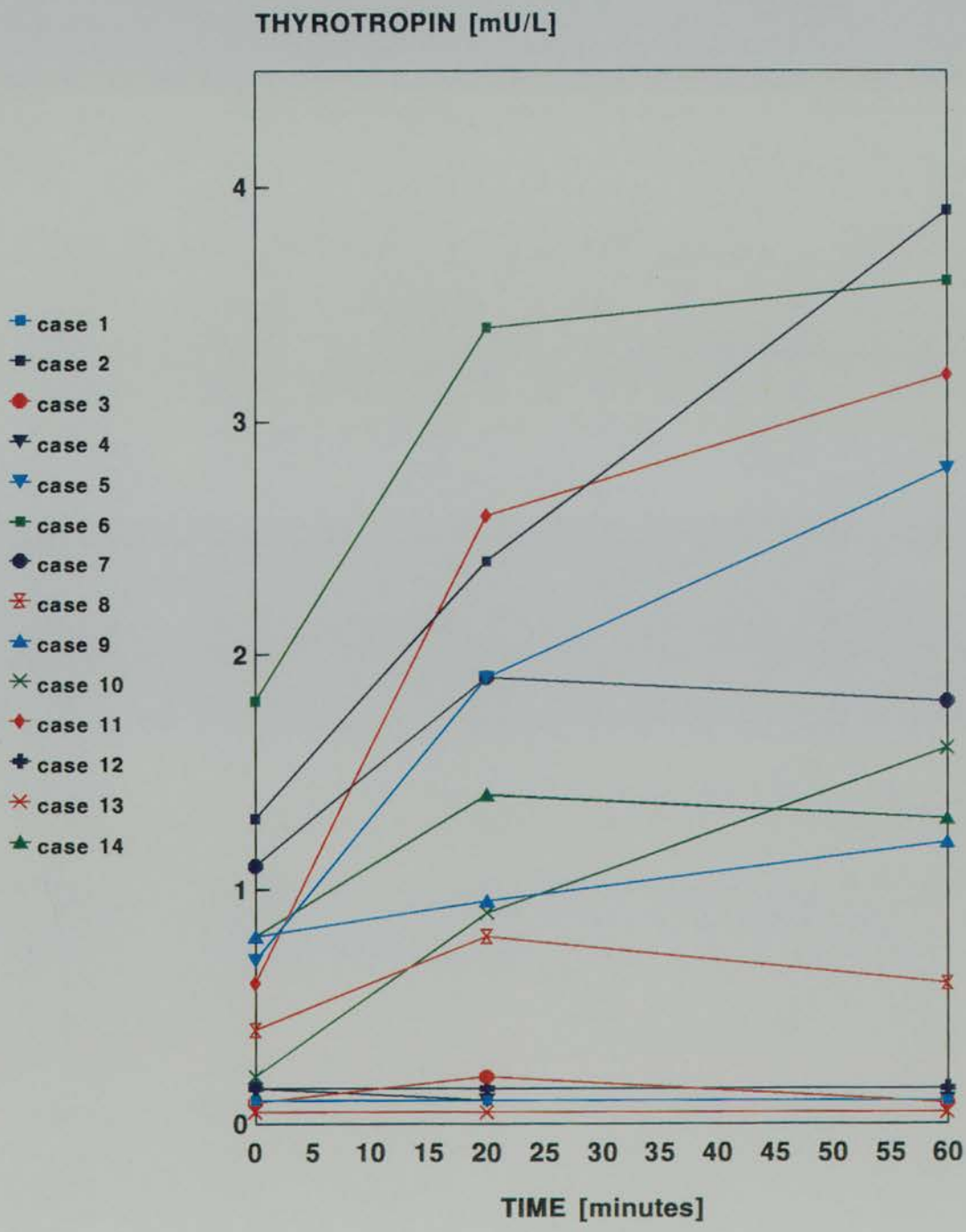


FIG 6.12. THYROTROPIN RESPONSE TO TRH TESTING IN ICU CASES

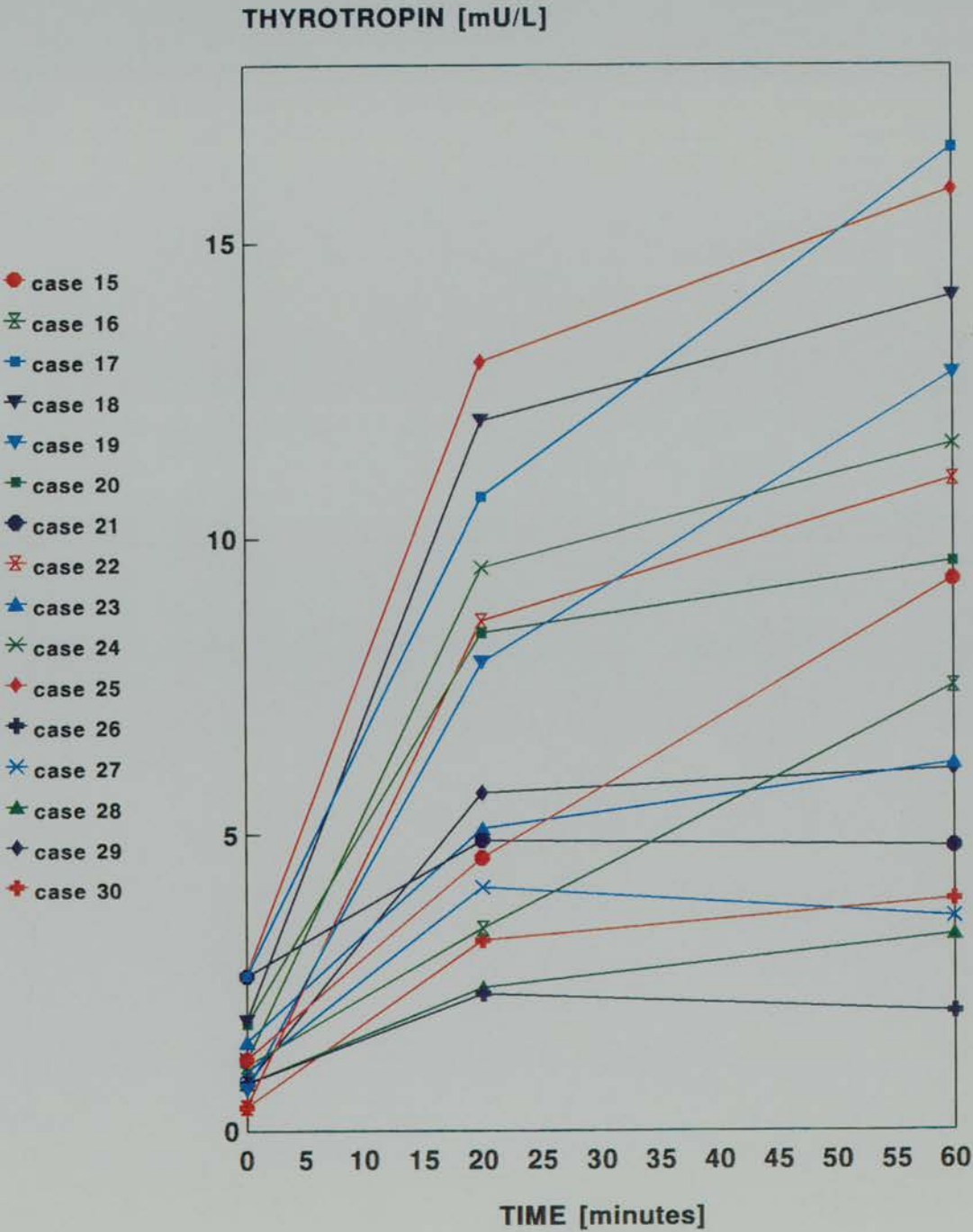


FIG 6.13. THYROTROPIN RESPONSE TO TRH TESTING
IN CONTROLS

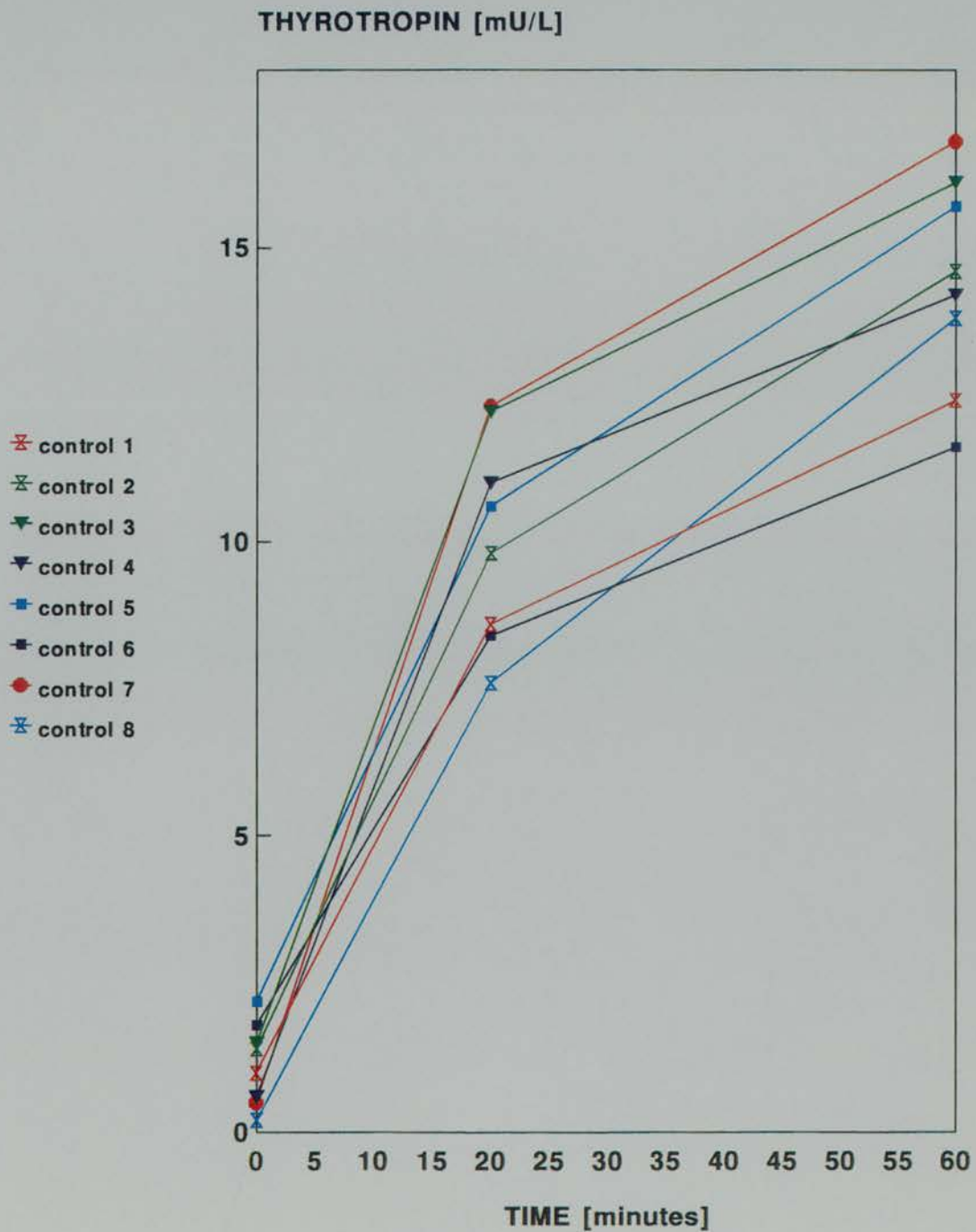


FIG 6.14. THYROXINE AND TRIIODOTHYRONINE RESPONSES TO A THYROTROPIN STIMULATION TEST IN CONTROLS

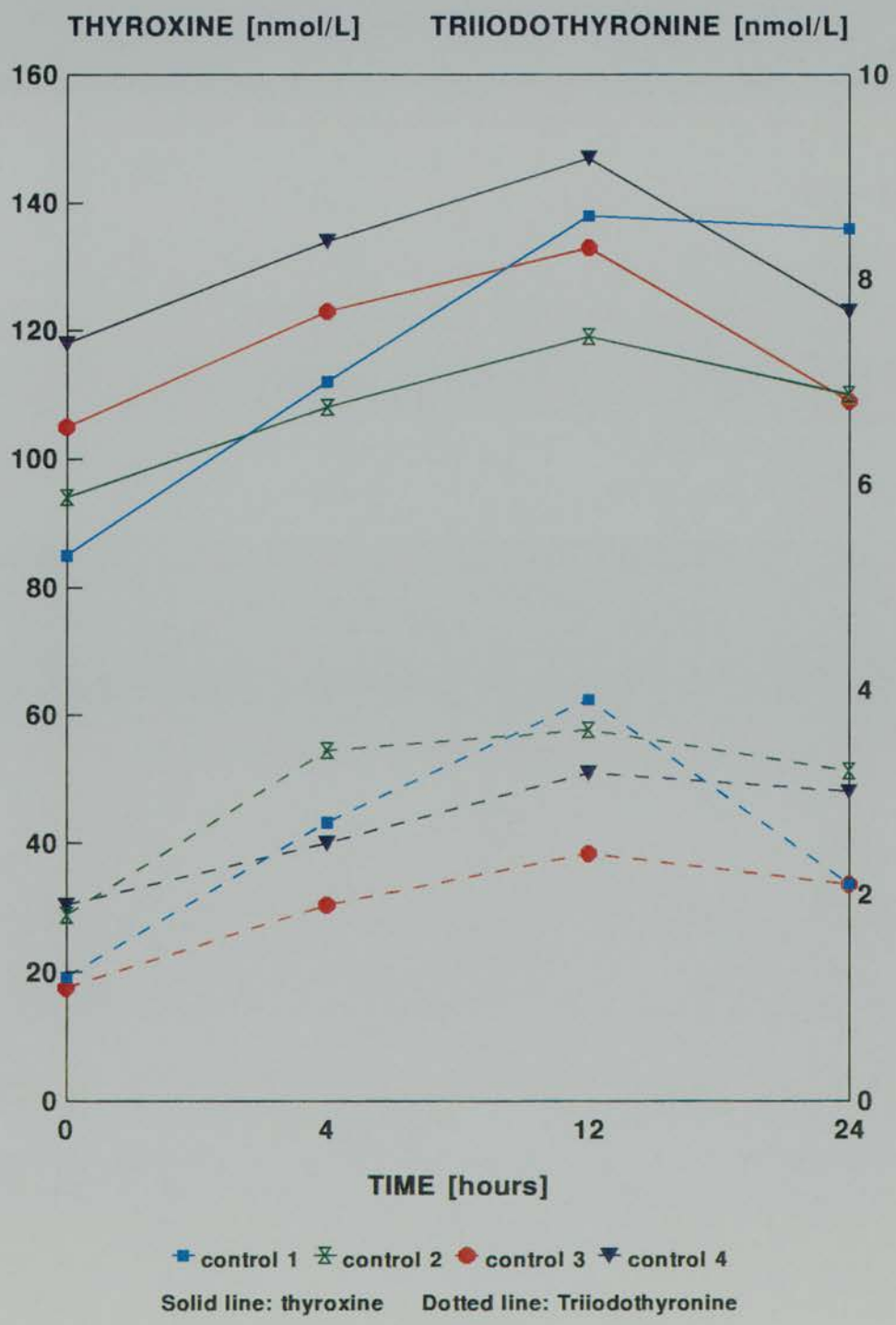


FIG 6.15. THYROXINE AND TRIIODOTHYRONINE RESPONSES TO A THYROTROPIN STIMULATION TEST: GROUP ONE

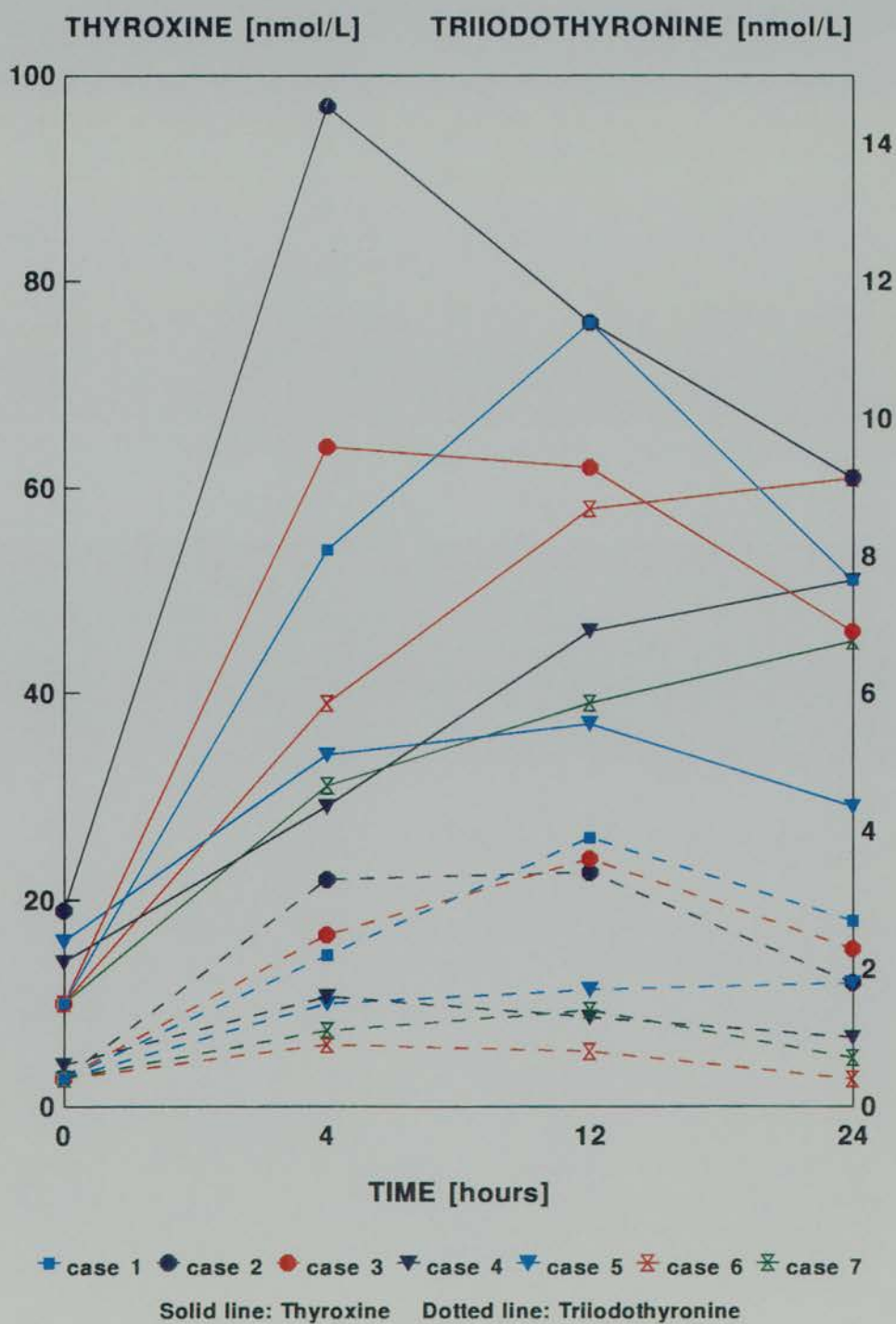
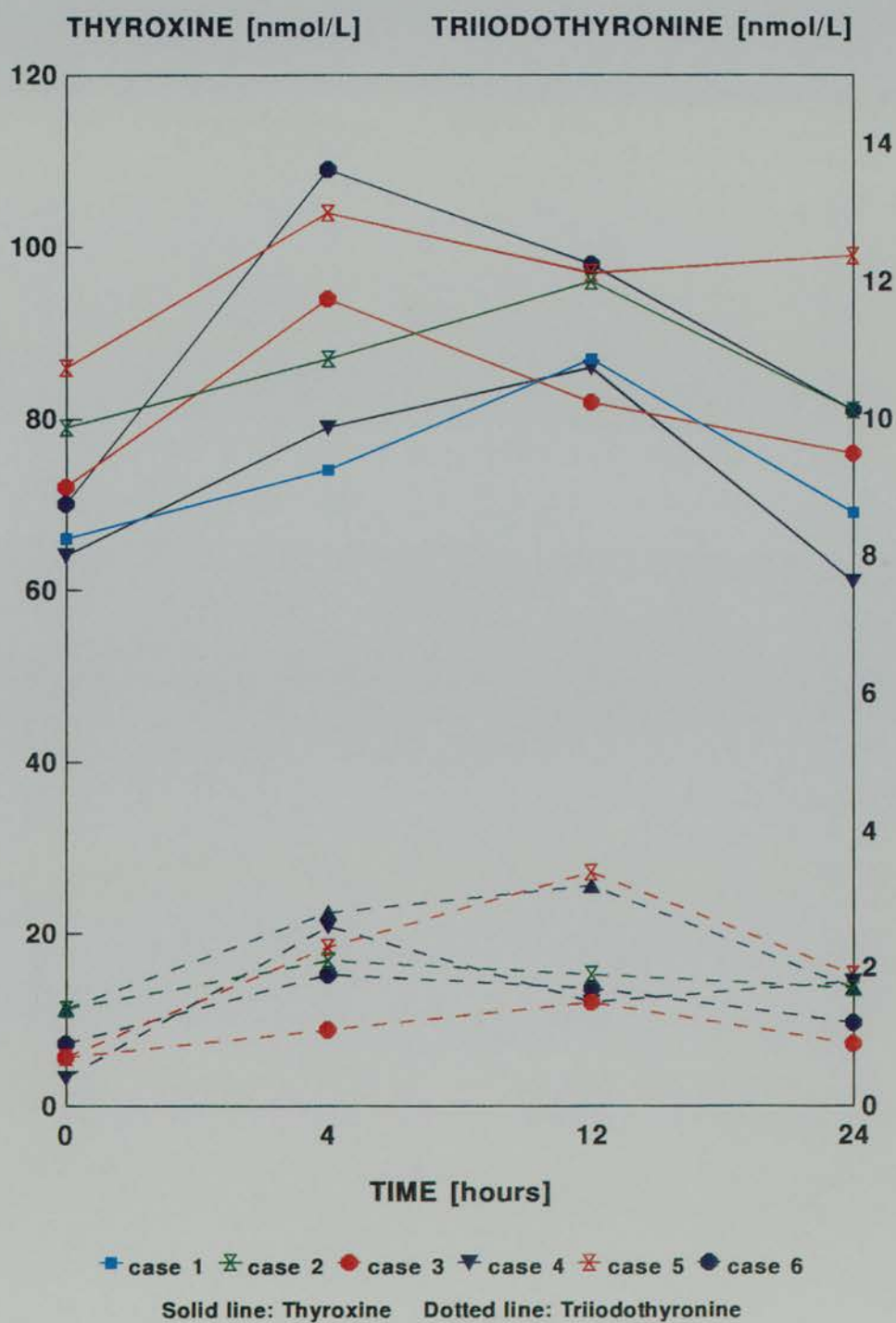


FIG 6.16. THYROXINE AND TRIIODOTHYRONINE RESPONSES TO A THYROTROPIN STIMULATION TEST: GROUP TWO



Discussion

TSH Concentration, Severity of Illness and Mortality

The majority of TSH concentrations in this study were within the reported working range of the assay. However, 15% of cases had subnormal TSH concentrations. How should this be interpreted. Studies of the ability of modern TSH assays to identify cases of hyperthyroidism (Spencer et al, 1987; O'Hare et al, 1991) have demonstrated a high sensitivity but a relatively low specificity. In other words, a significant proportion of cases with subnormal TSH concentrations did not have hyperthyroidism, although an undetectable TSH level (<0.1 mU/L), using a sensitive immunoradiometric assay, was more specific. Studies of the range of TSH concentrations in other populations are detailed in Table 6.9. The prevalence of low TSH levels in the ICU-based studies was high, but a significant proportion of patients were receiving dopamine or steroids. There is no published data concerning the range of TSH concentrations in a large number of severely ill patients not receiving dopamine or steroids.

Chosich et al (1989) demonstrated a low prevalence of subnormal TSH levels in a heterogeneous group of hospital patients [Table 6.9]. On repeat sampling, 80% of these cases still had subnormal concentrations. The causes of subnormal TSH levels were treated hyperthyroidism [35%], thyroxine replacement therapy [12%], euthyroid multinodular goitre [17%], pituitary disease [8%] and acute illness [28%]. Excluding patients with pituitary or thyroid disease, the

prevalence of subnormal TSH levels fell to 1.2%. Exclusion of cases with biochemical hyperthyroidism from the study by Arem et al (1990) gave a prevalence of subnormal TSH levels of 4.1% in outpatients and 5.6% in inpatients. However, this was a retrospective study, and the proportion of patients with a history of pituitary or thyroid disease, or receiving steroids, dopamine, or thyroxine replacement therapy was not given.

Table 6.9. Previous studies of TSH concentration, measured using a sensitive immunoradiometric assay, during illness.

STUDY	TYPE [number] OF PATIENTS	NORMAL RANGE [mU/L]	% ABNORMAL VALUES (95% CI)	
			LOW	HIGH
Wehmann et al (1985)	ICU (35)	0.3-5.0	31 [17-49]	0 [0]
Chosich et al (1989)	Hospital (1400)	>0.3	4.3 [3.3-5.5]	3.8 [2.8-4.9]
Arem et al (1990)	Outpatients (267)	0.3-5.0	11.6 [8-16]	5.6 [3.2-9]
"	Inpatients (411)	0.3-5.0	8.5 [6-11.6]	6.8 [4.6-10]
"	ICU (37)	0.3-5.0	16 [6-32]	22 [10-14]
Spencer et al (1987)	Hospital (1580)	0.4-5.0	23 [21-25]	12 [10-14]
Boles et al (1987)	ICU (34)	0.4-5.0	38 [22-56]	0 [0]

On the basis of the published evidence it seems likely that the prevalence of subnormal TSH levels in hospital populations, excluding patients with known thyroid disease, is in the region of 1-5%. The 15% [95% CI = 10.7-19.3] prevalence of subnormal TSH concentrations in this study is unlikely to be due solely to an high incidence of undiagnosed hyperthyroidism or multinodular goitre. However, although individual TSH assays are reported to have low coefficients of variation, Piketty et al (1987) found that disagreement between different immunoradiometric TSH assays kits at subnormal and low normal concentrations was much greater. This must undermine the validity of low TSH concentrations measured using a single assay. On the other hand, the negative correlation between TSH concentration and APACHE II scores in this study, and the association between low TSH levels and high mortality, suggest that a low TSH level has clinical validity.

Why TSH levels correlate so strongly with severity of illness is unclear. There was no correlation between TSH levels and the potential confounding factors such as recent surgery, time of day, nutritional state and renal failure, when the effect of severity of illness was taken into account. The inverse correlation between plasma cortisol concentration and TSH concentration suggests that the very high concentrations of cortisol found during severe illness may suppress TSH synthesis and/or secretion.

The prevalence of raised TSH levels in this study was 13.5% [95% CI = 9.3-17.6]. There is no published work detailing

the frequency of increased TSH concentration in a large group of critically ill patients, with no history of thyroid disease. No raised TSH concentrations were found by Boles et al (1987) in 34 ICU patients or Wehmann et al (1985) in 35 severely ill patients undergoing bone marrow transplantation. However, patients in both studies received steroids and dopamine. Increased TSH concentrations have been reported in small numbers of patients on recovery from illness (Bacci et al, 1982; Hamblin et al, 1986), but these findings coincided with cessation of dopamine therapy. Brent et al (1986) reported 3 critically ill patients with TSH concentrations in the hypothyroid range [> 20 mU/L], in whom investigation revealed high normal thyroid hormone levels and a normal response to TRH. Wong et al (1981) reported two similar cases. On the basis of this evidence, Spencer [1988] argued that raised TSH concentrations during acute illness do not necessarily indicate hypothyroidism. This is confirmed by the findings in this thesis. Indeed, patients with raised TSH concentrations had the highest concentrations of total T₄, and raised TSH concentrations tended to fall on recovery from illness. It has been suggested that high TSH levels indicate recovery from illness and precede the increase in T₃ and T₄ levels (Bacci et al, 1982; Spencer et al, 1987). However, in this study, high TSH levels were found early in the evolution of the illness.

The high mortality in cases with low TSH is not unexpected given the correlation between TSH and severity of illness. The association of high TSH levels with a very low mortality

is more difficult to explain. This prognostic value of TSH concentration in critical illness has not been reported previously.

TRH Tests

TRH tests are useful in determining the significance of isolated low TSH concentrations. The expected response to TRH is an increment in TSH concentration of between 5 and 30 mU/L. In two thirds of the ICU patients studied, the TSH increment was below 5 mU/L, and there was virtually no overlap in TSH increment between cases and controls. Impairment of TSH responsiveness to TRH has been documented previously during illness [Table 6.10].

Table 6.10. Details of previous studies of TSH responsiveness to TRH stimulation tests during illness, including whether or not patients studied were receiving treatment with dopamine or steroids.

Study	PATIENTS	N	MEAN [SD] TSH INCREMENT	DOPAMINE OR STERIODS
Maturlo et al (1980)	Medical	15	3.8 [0.5]	No
Vierhapper et al (1982)	ICU	16	4.4 [1.8]	Yes
Becker et al (1982)	Burns	10	9.5 [4.6]	No
Quint et al (1985)	ICU	13	8.0 [5.6]	DK
Boles et al (1987)	ICU	34	5.1 [0-14]*	DK

* median [range]

A number of other studies gave only qualitative results of TRH tests. Heinen et al (1980) investigated 7 ICU patients

with low T4 levels and found the TSH response to TRH to be "minimal" or absent in all 7 cases. However, all cases were treated with high doses of dopamine and steroids. Similarly impaired responsiveness in dopamine-treated ICU cases was noted by Faber et al (1987). Some studies have reported no impairment of TSH response to TRH during illness. Talwar et al (1976) found a mean [SD] TSH increment following TRH of 14.3 [2.1] mU/L in 12 febrile patients suffering from infections. Boles et al (1987) found that severely ill patients with subnormal basal TSH levels had near normal responses to TRH.

The present study suggests that pituitary responsiveness to TRH is impaired in the majority of severely ill patients, and absent in a significant proportion. Impaired TSH responsiveness has been reported in renal failure (Czernichow et al, 1976), and as a consequence of fasting (Borst et al, 1983), but there was no association between renal dysfunction or absence of nutritional replacement and impaired TSH responsiveness in this study. Patients with a poor response to TRH tended to have low basal TSH and total T4 concentrations raising the possibility that in some patients reduced TSH secretion might contribute to the low circulating thyroid hormone levels.

TSH Tests

TSH stimulation tests during illness have not been reported previously. The increases in thyroid hormone concentrations in controls were within the expected range. The finding that

patients with low basal T4 and TSH concentrations and a poor response to TRH had the highest increments in thyroid hormone levels following TSH is interesting. Interpretation of increased thyroid responsiveness to TSH in critically ill patients with apparent impairment of TSH secretion is difficult, but the finding is not inconsistent with the hypothesis that reduced TSH secretion may contribute to the low concentrations of thyroid hormones in some critically ill patients. Furthermore, it suggests that function of the thyroid gland itself is not commonly impaired during critical illness, even in patients with otherwise widespread organ failure.

Thyroid Hormone Concentrations

The T4 and T3 concentrations found in this study are consistent with previous studies of thyroid function in critical illness [Table 6.11]. However, the comparatively small size of previous studies has not allowed accurate definition of the expected range of T3 and T4 concentrations during illness, and although hormone levels have been shown to fall as illness severity increases, the exact relationship has not been defined in terms of APACHE II scores.

In addition to the work outlined in Table 6.11, 2 further studies of thyroid hormones should be mentioned. Song et al (1991) investigated 133 severely ill patients and found that 60% had a T4 concentration below 78 nmol/L, 53% had T3 concentrations below 0.9 nmol/L, and that there was a strong association between low T4 levels and a poor outcome. Ham-

blin et al (1986) investigated 60 ICU patients and found that 24 [40%] had T4 levels below 35 nmol/l. In the study by Slag et al (1981), 19 out of 86 [22%] ICU patients had T4 levels of less than 40 nmol/L, and this group had an 84% mortality compared with a 15% mortality among patients with T4 levels within the normal range.

Table 6.11. Previous studies of thyroid hormone concentrations in severe illness.

STUDY	TYPE (NUM) OF CASES	THYROXINE [nmol/L]		TRIIODOTHYRONINE [nmol/L]	
		MEAN [SD]	RANGE	MEAN [SD]	RANGE
Slag et al (1981)	ICU (86)	89.7 [16]	<38-150	n/a	n/a
Bacci et al (1982)	ICU (51)	52.8 [19]	n/a	0.50 [0.3]	n/a
Kaplan et al (1982)	Medical (14)	53.4 [12]	37-72	n/a	n/a
Baue et al (1984)	ICU (25)	54.2 [26]	n/a	0.85 [0.4]	n/a
Surks et al (1988)	ICU (30)	68.2 [24]	13-131	1.25 [0.14]	n/a

The 95% ranges of T4 concentration defined in this study for different degrees of illness severity might be of some use to clinicians. For example, a T4 concentration of 10 nmol/L in a patient with an APACHE II score > 24 is not unexpected,

whereas the same concentration in a patient with an APACHE II score of 16-24 would be subnormal, and the same concentration in a patient with an APACHE II < 16 would certainly require further investigation. These ranges might therefore be of some value in determining which critically ill patients have the Sick Euthyroid Syndrome and which if any might have preexisting thyroid disease.

The prognostic value of T4 levels has been documented previously, and will be discussed in Chapter 7. As expected mortality increases as total T4 concentration falls. However, total T4 concentration correlated inversely with APACHE II scores, and the mortality among patients with very low concentrations of total T4 was no greater than would be expected on the basis of severity of illness alone. In other words, very low total T4 levels do not appear to be detrimental in themselves. The implications of this finding for thyroid replacement during critical illness will be discussed in Chapter 8.

The distribution of T3 concentration was as expected. There was a very high incidence of the low T3 syndrome with 70% of patients having subnormal T3 concentrations, and nearly 60% having levels below assay sensitivity. This is in keeping with the findings of the previous studies (Chapter 5). The high incidence of low T3 levels meant that it was not possible to define any distinct ranges of T3 concentration based on illness severity. For the same reason T3 has less prognostic value than T4.

Thyroid Hormones and Metabolic Rate

It is frequently postulated that the "purpose" of the sick euthyroid syndrome is to limit metabolic rate and protein catabolism, which are stimulated during illness by the high concentrations of cortisol and catecholamines (Wartofsky and Burman, 1982; Chopra et al, 1983; Wehmann et al, 1985). This teleological argument is based on the similar changes in thyroid hormone metabolism which occur during starvation. There is, however, very little published work concerning the relationship between thyroid function and metabolic rate during illness.

Critically ill ventilated patients have an increased energy expenditure which is usually about 30 - 40 % in excess of the estimated basal metabolic rate (Carlsson et al, 1984; Lanschott et al, 1987; Soop et al, 1989). The findings of this study are in agreement with this, the majority of patients studied having an EMR of between 20 and 50%.

Estimation of metabolic rate using only carbon dioxide production and assuming a specific value for the respiratory quotient [RQ] is not ideal. Measurement of both carbon dioxide production and oxygen utilisation and the use of either a metabolic computer or the Weir equation to derive metabolic rate is more accurate (Forsberg et al, 1991; Kemper et al, 1992). However, there appears to be remarkably little variation in RQ during severe illness (Forsberg et al, 1991), and assuming an RQ of 0.8, although introducing a small error, is unlikely to lead to any bias towards or away from a relationship between thyroid status and metabolic

rate. The high reproducibility of the measurement of carbon dioxide production is encouraging, and the very similar EMR results to previous studies suggest that the method used was adequate. Indeed, variability in the conditions of measurement are likely to be more of a problem. Minor intensive care activities, such as passive physiotherapy increase metabolic rate significantly (Weissman et al, 1984). In this study, carbon dioxide production was measured under strict pre-specified conditions.

The correlation between EMR and body temperature is expected. In normal subjects, energy expenditure increases by 10 - 13% for each 1°C increase in body temperature. There was no correlation between T4 and temperature, suggesting that the relationship between EMR and T4 was not confounded by temperature. Confounding by a consistent effect of thyroid hormone concentration on RQ, and hence, a consistent bias in the estimation of metabolic rate is also unlikely. Confounding by sedative or inotropic drugs is possible. Sedation reduces metabolic rate (Robertson et al, 1984; Swinamer et al, 1988) and might lead to a bias. However, the ICU has a standard sedation policy, and there is unlikely to have been sufficient variation in sedative dosage to introduce significant bias. High doses of inotropes are likely to increase energy expenditure, and tend to be used in more severely ill patients. A confounding effect of inotropes cannot be excluded.

The inverse correlation between total T4 concentration and EMR argues against any clinically significant hypothyroidism

in patients with low total thyroid hormone levels. It would be consistent with the hypothesis that the changes in thyroid function during severe illness are, at least in part, a response to excessive metabolic rate. However, as T4 and EMR both correlate with severity of illness, as measured by APACHE II scores, the association between T4 and EMR may be confounded by other factors also related to illness severity. Moreover, the lack of any information about free thyroid hormone levels makes interpretation of the changes in metabolic rate in relation to the Sick Euthyroid Syndrome difficult.

(6) Conclusions

The aims of this section of the thesis were limited. The question of whether or not some severely ill patients may be biochemically hypothyroid was not addressed. Despite the lack of measurements of free thyroid hormone, a number of interesting findings have emerged. The main conclusions are as follows:

- 1) In patients without known preexisting thyroid disease, not receiving treatment with dopamine or steroids, the range of TSH concentrations found during illness is broader than in health. In approximately 15% of patients, TSH concentrations are subnormal, and in a third of these the TSH concentration is less than 0.1 mU/L. In approximately 13% of patients, TSH concentrations are raised during illness, de-

spite relatively normal total T4 levels. In the majority of cases, TSH concentrations return to normal on recovery. There is a strong inverse correlation between TSH concentration and severity of illness, with different normal ranges at different levels of illness. There is also a strong inverse correlation between TSH concentration and plasma cortisol concentration.

2) The TSH response to a TRH test is reduced or absent during critical illness. The response correlates positively with T4 concentration and inversely with APACHE II score and plasma cortisol concentration.

3) There is a positive correlation between the concentration of TSH and total thyroxine. Patients with low concentrations of both hormones have a subnormal or absent TSH response to TRH and an increased thyroid hormone response to TSH. These findings suggest that pituitary function may be impaired or suppressed during critical illness whereas the thyroid gland appears to function well. Low TSH concentrations may contribute to the changes of the Sick Euthyroid Syndrome.

4) Metabolic rate is increased in the majority of critically ill patients. There is an inverse correlation between metabolic rate and total thyroxine concentration.

5) Mortality increases as total T4 levels fall. However,

patients with low total T4 concentrations do not have a higher mortality than would be expected on the basis of their severity of illness. Taken together with the inverse correlation between total T4 concentration and metabolic rate, this casts doubt on the suggestion that critically ill patients with low total thyroid hormone concentrations might benefit from hormone replacement.

Directions for Future Research

There are a number of questions which would be interesting to answer. More detailed investigation of pituitary function during illness would be worthwhile. Are the low concentrations of TSH and impaired responsiveness to TRH due to the effects of cytokines on thyrotropic cells or due to raised concentrations of free thyroxine? Concentrations of FSH and LH are reduced during severe illness. Is there evidence of more widespread pituitary suppression or impairment? What is the TSH and thyroid hormone response to treatment with the monoclonal anti-cytokine antibodies now used in clinical practice? Given the frequency of widespread organ failure during critical illness, is there any pathological evidence of damage to the pituitary gland?

Chapter 7

Prognostic Value of Thyrotropin, Thyroid Hormones and Cortisol During Critical Illness

- (1) Introduction**
 - (2) Aims**
 - (3) Methods**
 - (4) Results**
 - (5) Figure**
 - (6) Discussion**
-

Introduction

There have been many pleas for rationalisation of the use of intensive care, which is expensive and can unnecessarily prolong the process of dying. Provision of intensive care accounts for between 10 and 20% of hospital expenditure (Birnbaum, 1986), and survival is inversely related to cost of treatment and length of stay (Detsky et al, 1981). At least 15% of ICU patients are admitted with underlying conditions from which there is no likelihood of survival, and die on the ICU, or a short time after discharge (Knaus et al, 1984). Most clinicians recognise the problem, yet are

reluctant to make the decision to withhold or withdraw intensive care from those too ill to benefit (Singer et al, 1983; Editorial, Lancet, 1985). Prognostic uncertainty appears to be an important factor in determining the expenditure of resources in the ICU (Detsky et al, 1981). Although it is recommended that predictions of a patient's prognosis influence triage decisions (CDC, 1983), physicians' predictions of outcome are frequently wrong (Kruse et al, 1988; Brannen et al, 1989). Different physicians caring for the same patient often disagree about the likelihood that the patient will survive (Poses et al, 1989), and often take a more optimistic view of the survival chances of patients under their own care than similar patients cared for by other clinicians (Poses et al, 1991). The accurate prediction of patient outcome has become a major objective of intensive care clinicians, and several prognostic scores based on estimates of illness severity have been developed (Knaus et al, 1985; Le Gall et al, 1984; Chang, 1989).

The most widely used prognostic system is the APACHE II system. When first developed the APACHE score contained 33 variables, each measuring physiological derangement. The APACHE II score was subsequently developed, based on 12 of the original 33 variables. Other similar scores in widespread use include the Mortality Prediction Model (Limeshow et al, 1987; Teres et al, 1987) and the Simplified Acute Physiology Score (Le Gall et al, 1984). Each of these models measures the deviation from normal of selected physiological parameters and equate loss of homeostasis with severity of

illness. However, prognosis is not always strongly correlated with physiological derangement. Diabetic ketoacidosis is associated with gross physiological abnormalities, but with correct treatment has a mortality of less than 5% (Wagner et al, 1986). There is a wide variation, 7 - 56%, in the observed death rates of patients with failure of 3 or more organ systems, suggesting that a number of factors may be important in determining outcome.

Prognosis is dependent on at least 4 factors, in addition to illness severity: (1) disease type, (2) age, (3) prior health status, and (4) the therapy available. Each of these must be considered in the step between measurement of illness severity and predicting outcome. The APACHE system and the other commonly used scores take each of these factors into account. Many other prognostic systems have been developed in recent years, including scores based on the number of organ systems failing (Fagon et al, 1993), electrolyte imbalance (Broner et al, 1990) and gastric pH (Doglio et al, 1991). However, despite much refinement, no score has proved accurate enough to influence clinical decision making in individual patients (Schaffer et al, 1993). At the present time prognostic scores are used to compare groups of ICU patients, either in research or audit, although, even their validity in this role has been questioned (Boyd and Grounds, 1993)

Although, prognostic scores do not predict individual outcome accurately, there is evidence that routine use of a prognostic system does influence the "intensity" of manage-

ment (Murray et al, 1993). Physicians are being encouraged to continue to study measures of illness severity and to develop new prognostic systems (Knaus, 1993). Alternative approaches to the measurement of severity of illness are needed. One possibility is the endocrine response to the physiological stress of critical illness. Certain individual endocrine parameters have been shown to predict outcome in critical illness (Slag et al, 1981; Schein et al, 1990; Span et al, 1992), but there has been no attempt to develop a prognostic index using a number of different endocrine parameters measured at a uniform time in a large number of intensive care patients.

(2) Aims

The aim of this study was to determine the prognostic value of an index combining thyroid hormones, TSH and cortisol, in patients not receiving hormone replacement or drugs which alter endocrine function.

(3) Patients and Methods

The 260 patients investigated were described in Chapter 2. Briefly, they formed 90% of all ICU admissions over a 2 year period. Of the 29 patients excluded, 21 were receiving dopamine on admission, which alters thyroid and pituitary function, 5 were on longterm steroid therapy, and 3 were receiving T4 replacement. The average age of patients stu-

died was 59.6 years [range 14-86] and there was a slight excess of males, 137 vs 123 females.

All patients were investigated on admission to the ICU. Blood was taken within 1 hour of admission, prior to treatment with dopamine, and stored at 4°C. A plasma sample was obtained for cortisol assay, and serum samples were taken for measurement of T4, T3 and TSH. All samples were transferred to the clinical chemistry laboratory within 24 hours. APACHE II scores were calculated using data gathered during the first 24 hours of admission. Outcome was measured at hospital discharge.

Cox's multiple logistic regression analysis was performed to define and weight the variables which were independently predictive of outcome. Statistical analysis was performed using a commercial software package (SPSS/PC+, version 3.0, SPSS, Chicago, USA).

(4) Results

Overall mortality was 31%, and median duration between ICU admission and death was 10 days (range, 2 hrs - 69 days). The overall mean (SD) APACHE II score was 19.8 (8.6) with mean (SD) predicted mortality 38% (19.6%).

The concentrations of each of the hormones measured and APACHE II scores differed significantly between survivors and nonsurvivors (Table 7.1). A multiple logistic regression analysis showed that of the hormones measured only cortisol, T4 and TSH were independent predictors of outcome. The

logistic model based on SI units was $P = 1/[1 + \exp(0.174 \text{ TSH} + 0.044 \text{ T4} - 0.0015 \text{ cortisol} - 0.51)]$. In standard American units: $P = 1/[1 + \exp(0.174 \text{ TSH} + 0.568 \text{ T4} - 0.042 \text{ cortisol} - 0.51)]$, where P is the probability of death. The predictive power of this Endocrine Index, measured by the area under the receiver operating curve [ROC], was 0.94 (95% CI = 0.91 - 0.96). The area under the APACHE II score ROC was 0.85 (95% CI = 0.81 - 0.89) indicating significantly lower predictive power (Figure 7.1).

At the 0.5 cut off point of the ROC both scores predict outcome with the greatest accuracy (Table 7.2). The Endocrine Index predicted outcome with 82% accuracy compared to 72% accuracy using APACHE II scores.

Table 7.1. Age, TSH, T4, T3, and cortisol concentrations, and APACHE II score with outcome predicted percentage mortality in survivors and nonsurvivors. Median and range unless stated otherwise.

	SURVIVORS	NONSURVIVORS
Number	172	88
Age [yrs]*	58.1 [15]	61.3 [13]
Thyrotropin; mU/L	2.2 [0.1-21]	0.7 [0.1-8.4] #
Thyroxine; mcg/dL	6.1 [<0.8-14]	2.9 [<0.8-10] #
Triiodothyronine; ng/dL	32 [<10-169]	13 [<10-91] #
Cortisol; mcg/dL	27 [5.6-89]	47 [7-134] #
APACHE II score*	17 [7]	26 [7] +
APACHE II outcome*	26 [14]	60 [19] +

* = mean [SD]. # = $p < 0.001$ (Wilcoxon's Rank Sum test).

+ = $p < 0.001$ (Student's t-test).

Table 7.2. Prediction of outcome at the 0.5 cut off point on the receiver operating curve for the Endocrine Index and APACHE II scores.

	OUTCOME PREDICTION				
	ENDOCRINE INDEX		APACHE II SCORE		TOTAL
	DIE	SURVIVE	DIE	SURVIVE	
OUTCOME					
DIED	59	29	35	53	88
SURVIVED	19	153	20	152	172
TOTAL	78	182	55	205	260
SENSITIVITY:	67%		40%		
SPECIFICITY:	89%		88%		
ACCURACY:	82%		72%		
+ve PREDICTIVE VALUE:	76%		63%		
-ve PREDICTIVE VALUE:	84%		74%		

Tables 7.3 and 7.4 detail the prediction of death by both scores at the upper and lower 4 cut off points of the ROC. The Endocrine Index has the greater accuracy than the APACHE II score at all points on the ROC. It predicts survival with greater than 90% specificity in 125 patients (73% of survivors), and predicts death with greater than 95% specificity in 55 patients (63% of deaths). Both scores predicted survival with greater sensitivity than they predicted death. An outcome prediction equation produced by multiple regression analysis performed using both the APACHE II score and the endocrine parameters did not predict outcome more accurately than the Endocrine Index alone (area under ROC = 0.94). Of the 48 incorrect outcome predictions by the Endo-

crine Index at the 0.5 cut off point, 41 were also predicted incorrectly by the APACHE II score at that cut off point (85% concurrence). Similarly of 29 patients predicted to survive by the Endocrine Index who subsequently died, 27 were predicted to survive by APACHE II scores (93% concurrence).

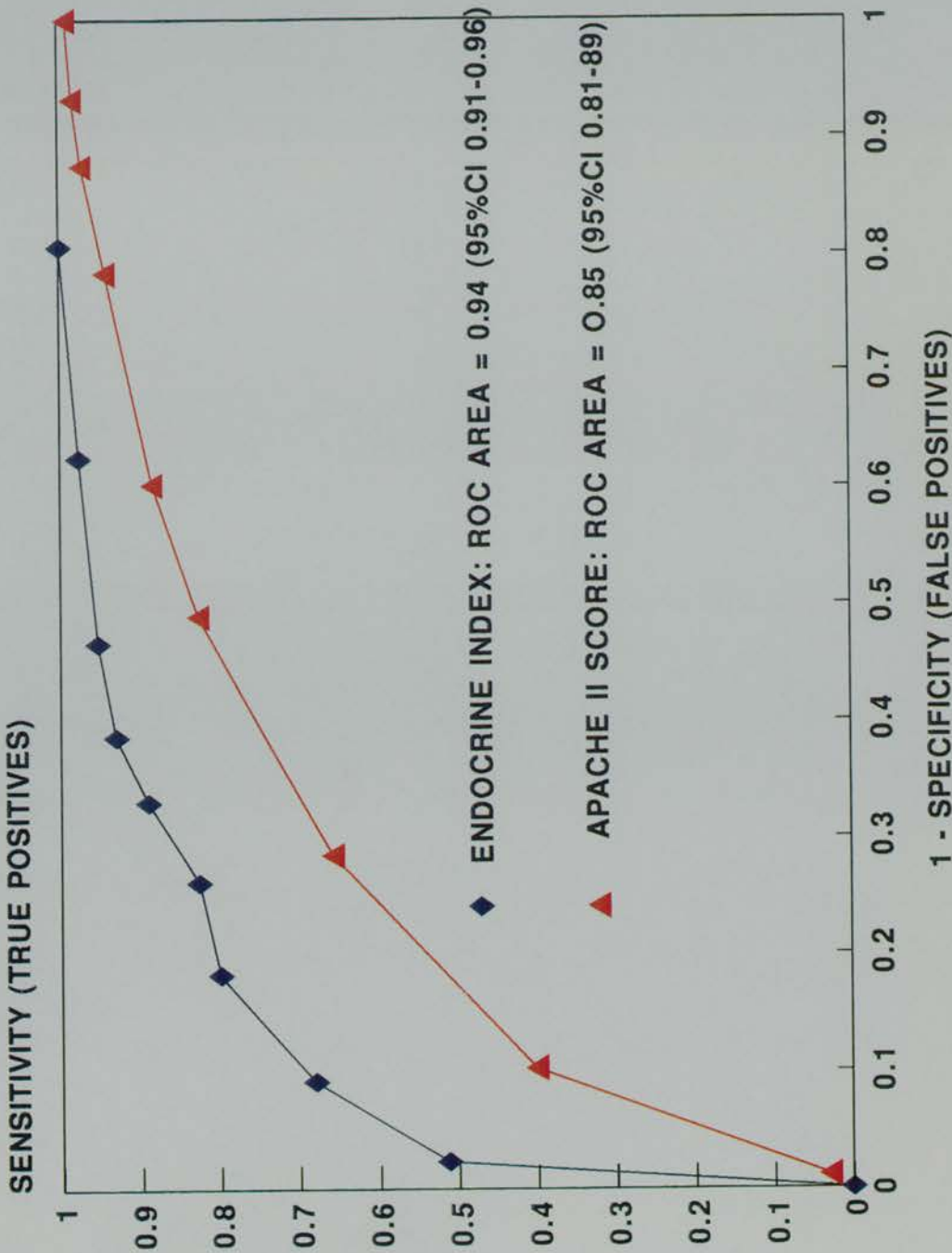
Table 7.3. Prediction of death by the Endocrine Index and APACHE II scores at the lower four points of the receiver operating curve.

CUT-OFF	OUTCOME	OUTCOME PREDICTION			
		ENDOCRINE INDEX		APACHE II SCORE	
		DIE	SURVIVE	DIE	SURVIVE
0.1	DIED	17	71	0	88
	SURVIVED	0	172	0	172
	SENSITIVITY =		19%		0%
	SPECIFICITY =		100%		100%
	ACCURACY =		73%		66%
0.2	DIED	33	55	6	82
	SURVIVED	4	168	3	169
	SENSITIVITY =		38%		7%
	SPECIFICITY =		98%		98%
	ACCURACY =		77%		67%
0.3	DIED	47	41	11	77
	SURVIVED	8	164	5	167
	SENSITIVITY =		53%		13%
	SPECIFICITY =		95%		97%
	ACCURACY =		81%		68%
0.4	DIED	54	34	19	69
	SURVIVED	12	160	10	162
	SENSITIVITY =		61%		22%
	SPECIFICITY =		93%		94%
	ACCURACY =		82%		70%

Table 7.4. Prediction of survival by the Endocrine Index and APACHE II scores at the top four points of the receiver operating curve.

CUT-OFF	OUTCOME	OUTCOME PREDICTION			
		ENDOCRINE INDEX		APACHE II SCORE	
		SURVIVE	DIE	SURVIVE	DIE
0.6	SURVIVED	142	30	142	30
	DIED	23	65	43	45
	SENSITIVITY =	83%		83%	
	SPECIFICITY =	74%		51%	
	ACCURACY =	80%		72%	
0.7	SURVIVED	134	38	113	59
	DIED	16	72	25	63
	SENSITIVITY =	78%		66%	
	SPECIFICITY =	82%		72%	
	ACCURACY =	75%		68%	
0.8	SURVIVED	117	55	69	103
	DIED	8	80	9	79
	SENSITIVITY =	68%		40%	
	SPECIFICITY =	91%		90%	
	ACCURACY =	76%		57%	
0.9	SURVIVED	88	84	5	167
	DIED	2	86	1	87
	SENSITIVITY =	51%		3%	
	SPECIFICITY =	98%		99%	
	ACCURACY =	67%		35%	

FIG 7.1. PREDICTIVE POWERS OF THE ENDOCRINE INDEX AND APACHE II SCORES AS MEASURED BY AREA UNDER THE ROC CURVE



(6) Discussion

The changes in thyroid and adrenocortical function which accompany critical illness have been detailed in earlier chapters. Low concentrations of TSH and total T4, and high concentrations of cortisol have been shown to indicate a poor prognosis. The value of a combined endocrine prognostic index has not previously been fully assessed. The results of this study demonstrate that the response of thyroid and adrenal function to critical illness is of significantly greater prognostic value than APACHE II scores.

None of the prognostic scores devised for use in ICU patients have proved accurate enough to influence clinical decision making in individual cases (Schaffer et al, 1993). The reason for this is that the predictions lack specificity. Patients predicted to die, often survive, and patients predicted to survive, often die. The scores do not therefore help ICU clinicians allocate resources to patients most likely to benefit. At least in terms of specificity of prediction, the Endocrine Index is an improvement on some previously published scores [3-5]. The index predicted death with 100% specificity in 17 (20%) nonsurvivors, and 98% specificity in 37 (42%) nonsurvivors. It predicted survival with 98% specificity in 90 (52%) survivors. This is far from perfect, but the high specificity of prediction in significant numbers of patients is encouraging. Outcome prediction using APACHE II scores achieved high specificity in very few patients.

It is interesting that combining APACHE II scores with the

endocrine parameters did not improve outcome prediction. The high concurrence of incorrect predictions is unexpected with two scores based on such different data. It is also noteworthy that both the Endocrine Index and APACHE II scores predicted survival with greater sensitivity than they predicted death. The accuracy of both scores was limited by the same unexpected deaths. This apparent flaw in the predictive power of physiological scoring systems requires further consideration.

Survival depends on the maintenance of homeostasis. By measuring loss of homeostasis, physiological scoring systems predict survival with a high sensitivity. Prediction of death is less sensitive. This is probably because causes of death in ICU patients, such as myocardial infarction, pulmonary embolus, gastrointestinal haemorrhage etc, although induced by illness, may not be closely related to illness severity. These "unexpected" deaths in patients with relatively well maintained homeostasis will impair the sensitivity of prediction of death by physiological scoring systems. This ability of physiological scores to highlight "unexpected" deaths might be helpful in directing audit. The death of a patient who was predicted to survive by two independent physiological scoring systems deserves detailed audit. Such patients will not have had severe multi-organ failure and their deaths should be regarded as having been potentially preventable.

The Endocrine Index requires validation on other cohorts of ICU patients. In its present form it predicted outcome with

98% specificity in about half of the patients. Further work is required to discover whether addition of other endocrine parameters to the index will improve prediction of outcome. However, any prognostic index based on measurement of illness severity will have limited accuracy because of the frequency of deaths in patients who do not have severe impairment of homeostasis. Using physiological scoring systems to direct audit might reveal preventable causes of "unexpected" death in ICU patients, and thereby potentially reduce ICU mortality.

Chapter 8

Implications of the Results of these Studies For Clinical Trials of Hormone Replacement During Critical Illness

- (1) Absolute Risk and Sample Size in Clinical Trials
 - (2) Implications for Trials of Steroid Replacement
 - (3) Implications for Trials of Thyroid Hormone Replacement
-

The randomised controlled clinical trial is the accepted methodology for the determination of the efficacy of medical treatments. Before a treatment can be tested in a clinical trial, there must be some evidence that the treatment has potential for benefit. Much of this thesis has been concerned with the question of whether or not there is an association between low hormone concentrations or a poor response to stimulation tests and mortality. Given the difficulties with interpretation of total circulating hormone concentrations during illness, any association with mortality must be viewed with caution and is not evidence of tissue insufficiency of these hormones. However, the findings in this thesis do have some useful implications for future clinical trials of hormone replacement in critical illness.

Large clinical trials with considerable statistical power

are often required in order to convince clinicians of the presence or absence of a treatment effect and to change clinical practice. Such trials are expensive, time consuming and rely heavily on the good will of participating patients and clinicians. In order to maximise the power and limit the size of initial trials, it is necessary to perform them in patients who appear most likely to benefit from the treatment. The relevance of the findings of the studies in this thesis to the determination of entry criteria and size of initial trials of hormone replacement during critical illness are discussed below. Firstly, a number of general principals are discussed.

(1) Absolute Risk and Sample Size in Clinical Trials

The number of patients required to test the null hypothesis in a clinical trial depends to some extent on the relative treatment effect. However, the absolute untreated risk of the trial outcome in the patients studied is of equal importance. The trials in Table 8.1 illustrate the fact that a significant result in a clinical trial is more dependent on the number of outcomes in the trial than the number of patients. In other words, the statistical power of a trial, for a given relative treatment effect, is determined mainly by the absolute untreated risk rather than the trial size. Thus, in Table 8.1, the significance of the smaller treatment effects in the higher risk patients is greater than the larger treatment effects in the lower risk patients. For example, a 15% treatment effect is significant in a trial

with 500 patients per group with an untreated risk of death of 50%, whereas a 20% treatment effect is non-significant in a trial with 1000 patients per group with an untreated risk of death of 10%.

Table 8.1. The significance at the 95% level of confidence of various clinical trials of treatments with different treatment effects on patients with different absolute risks of death without treatment.

GROUP SIZE (N)	ABSOLUTE RISK OF DEATH	RELATIVE RISK REDUCTION	ODDS REDUCTION (95% CI)
100	10%	50%	0.47 (0.16-1.44)
100	50%	25%	0.59 (0.33-1.03)
500	10%	30%	0.68 (0.43-1.06)
500	50%	15%	0.74 (0.57-0.94)
1000	10%	20%	0.78 (0.58-1.06)
1000	50%	10%	0.82 (0.69-0.98)

(2) Implications for Trials of Steroid Replacement

The finding that for a given level of illness severity, low total plasma cortisol concentrations are associated with an increased mortality is important from the point of view of clinical trials. Firstly, although neither the tissue availability nor the tissue requirement for cortisol during illness is known, the greater than expected mortality might possibly be due to the low concentrations of cortisol. Secondly, a trial of cortisol replacement in patients with

low cortisol concentrations and a greater than expected mortality will be attractive to participating clinicians. Thirdly, the high absolute risk of death without treatment means that a trial could be relatively small and yet still have the power to exclude a moderate treatment effect.

If trial eligibility was confined to patients with an admission plasma cortisol concentration below the normal range defined in this thesis for patients with APACHE II scores of <16 and 16-24, and below 500 nmol/L in patients with APACHE II scores >24, how many patients would have to be studied to detect a 20% reduction in mortality? Of the patients studied in this thesis, 17 (7%) would be eligible. The mortality amongst these patients was 77% (95% CI, 50-93). Assuming the lower limit of mortality of 50%, a trial with 200 treated cases and 200 controls would be required in order to detect a 20% reduction in mortality with treatment [Treatment group: 80 deaths/200 vs Control group: 100 deaths/200. Odds ratio = 0.67, 95% CI = 0.45 - 0.99]. If the mortality among these patients was 77%, then the number required would be reduced to 80 patients in each group [Treatment group: 50 deaths/80 vs Control group: 62 deaths/80. Odds ratio = 0.48, 95% CI = 0.24 - 0.97].

If the findings of this thesis were ignored and all ICU patients were studied, a much larger trial would be necessary in order to detect a 20% reduction in mortality. Overall mortality among the 260 patients in this study was 34% (95% CI = 28 - 40). If we assume a mortality of 34%, then a study with 500 patients in each group would be required to

detect a 20% reduction in mortality at the 95% level of confidence [Treatment group: 136 deaths/500 vs Control group: 170 deaths/500. Odds ratio = 0.73, 95% CI = 0.55 - 0.95]. However, the overall benefit of steroid replacement in all patients might well be less than in patients with low cortisol levels and a high mortality. If all patients were treated, a trial with 2000 patients in each group would be required in order to detect a 10% reduction in an untreated mortality of 34%.

The above calculations illustrate the effect of the type of patients studied on the sample size required. The disadvantage of selecting the small proportion of high risk cases is that they are few in number and accrual would therefore be slow. For example, assuming 100% patient cooperation, a trial with 80 high risk patients in each group would take 20 years to complete in the ICU in which this study was performed. However, a study of low risk patients with 2000 patients in each group would take 25 years to complete. Both studies would, of course, need to be multi-centre. Accrual into the high risk study would be increased by allowing serial measurement of plasma cortisol rather than a single admission measurement, with entry into the trial when plasma cortisol fell below the above limits.

Patients with a plasma cortisol response to a standard ACTH stimulation test of less than 250 nmol/L also had a higher than expected mortality in this study. The mortality among the 25 patients with a cortisol response of less than 250 nmol/L was 76% (95% CI = 55 - 91). In the 13 of these pa-

tients who were suffering from septic shock, mortality was 100% (95% CI = 75 - 100). This may indicate minor adrenocortical impairment due to widespread organ failure. The high mortality reflecting the widespread organ failure rather than any insufficiency of cortisol. However, as is illustrated above, because of the high mortality, clinically important insufficiency could be excluded by a trial of relatively small numbers of patients.

(3) Implications for Trials of Thyroid Hormone Replacement

Low concentrations of total thyroid hormones were not independently associated with a greater than expected mortality when severity of illness was taken into account. This suggests that low total thyroid hormone concentrations do not directly increase mortality. Moreover, those patients with the lowest total thyroid hormone levels had the highest metabolic rates. Total thyroid hormone levels give no information about tissue availability of thyroid hormone during severe illness. However, these results do not support the hypothesis that critically ill patients would benefit from thyroid hormone replacement.

The two published controlled trials of thyroid hormone replacement during critical illness in man involved 19 treated patients and 19 controls (Hesch et al, 1981; Brent et al, 1986). There were 12 deaths in each group (relative risk = 1.00, 95% CI = 0.62 - 1.63). The combined result of these trials is consistent with a treatment effect somewhere between a 40% improvement in survival and a 60% increase in

mortality. From the point of view of further clinical trials of thyroid hormone replacement during critical illness, the results of this thesis provide no evidence that any particular group of patients would be particularly likely to benefit.

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Appendix

A Protocol for the Investigation of Adrenocortical Function on an Intensive Care Unit

(1) Aims

(2) Methods

- (a) Venepuncture and central line insertion.
- (b) Intravenous fluids and blood transfusion.
- (c) Enteral fluids.
- (d) Chest physiotherapy.
- (e) Haemodialysis.

(3) Results

(4) Figures

(5) Discusssion

(6) Protocol

(1) Aims

The methodological problems of research involving critically ill patients were discussed in Chapter 1. Interpretation of results is hindered by numerous confounding variables related to monitoring and treatment. The aim of this section of the thesis was to determine which of the common ICU interventions alter plasma cortisol levels. The purpose being to develop a protocol for the performance of dynamic tests of adrenocortical function in an ICU environment.

(2) Methods

(a) Venepuncture and Central Line Insertion

Ten patients were studied in order to determine whether peripheral venepuncture alters plasma cortisol concentration. A blood sample was taken via a central venous line, and a 20 French Gauge cannula then inserted into an antecubital fossa vein and a further sample taken immediately following insertion of the cannula. Five patients were sedated and ventilated and 5 were conscious, unsedated and self-venti-

lating.

Twelve patients were studied in order to determine whether the insertion of a central venous line altered plasma cortisol concentration. A triple lumen central line was used in each case. All lines were inserted using the Seldinger technique and a subclavicular approach after infiltration of skin and underlying muscle with 5mls of 2% lignocaine. Samples were taken by peripheral venepuncture immediately before the line was inserted and at 30 and 60 minutes after the procedure commenced. Seven patients were sedated with intravenous propofol or midazolam and ventilated, and the remaining 5 were conscious, unsedated and self-ventilating.

(b) Intravenous Fluids and Blood Transfusion

Twenty four patients were investigated in order to determine the effects of intravenous fluid administration and blood transfusion on plasma cortisol concentration. All intravenous fluids were stopped for 2 hours prior to the study. A baseline blood sample was taken for plasma cortisol measurement and the specified intravenous fluid was given at a uniform rate over 2 hours. Six patients were given 500 ml of 0.9% saline, 6 were given 100 ml of 20% albumin, 6 were given 400 ml of whole blood, and the remaining 6 were given 500 ml of full strength total parenteral nutrition (1000 Kcal/l, 80% lipid). Six patients receiving intravenous saline at a rate of 62.5 ml/hour were used as controls. In the control patients fluids were not stopped prior to the study. All infusions were started at 1000h and were given via central venous lines or antecubital fossa cannulae. Further plasma cortisol measurements were made 1, 2 and 4 hours after starting the infusion. No potentially stressful procedures such as haemodialysis, physiotherapy or insertion of arterial or venous lines were performed during this period. Relatives of the patients were able to visit during the period and nurses were able to perform all procedures felt to be necessary, including oropharyngeal and tracheobronchial suction.

(c) Enteral Fluids

Twelve patients receiving enteral feeding were investigated. All patients were fed via a fine bore nasogastric tube. Feeding was stopped at midnight on the evening prior to the study. A baseline blood sample for plasma cortisol estimation was taken at 9 am. Fluid was then given via the nasogastric tube. Six patients were given 500 ml of full strength

enteral feed over 2 hours, and 6 were given 500 ml of water over the same period. Further plasma cortisol measurements were made 1, 2 and 4 hours after starting the infusion. The same restrictions on stressful procedures as for the study of IV fluids were adopted for the 4 hours of the study.

(d) Physiotherapy

Eight patients were investigated to determine the effect of chest physiotherapy on plasma cortisol levels. Blood was taken for a baseline cortisol level immediately prior to physiotherapy. Each patient underwent the physiotherapy regimen which was clinically indicated at the time. This included chest physiotherapy in all cases, and required tracheobronchial suction in the ventilated patients. All patients were investigated between 0900h and 1200h. Five patients were ventilated and sedated and the remaining 3 were conscious, self ventilating and unsedated. Physiotherapy lasted between 10 and 30 minutes. Further blood samples were taken 30 minutes and 1 hour after physiotherapy was commenced.

(e) Haemodialysis

Eight patients suffering from acute renal failure secondary to septic shock were investigated. Blood was taken for cortisol measurement immediately prior to beginning dialysis and 30 minutes, 1 hour, 2 hours and 4 hours after commencement of dialysis. In all cases dialysis was performed using an automated haemodialysis machine and a right subclavian vein double lumen dialysis catheter. A bolus intravenous injection of heparin 5000 units was given to all patients prior to dialysis. The flow rates of blood to the dialysis machine varied between 100 and 500 ml/min. Dialysis sessions began between 0900h and 1100h. Patients were used as their own controls, the protocol being repeated at the same time the following day without dialysis. Blood pressure was recorded during the dialysis and the control periods using measurements from a transducer in either a radial or dorsalis pedis artery. No potentially stressful events, such as physiotherapy or the insertion of arterial or venous lines, were allowed during the study period.

(3) Results

Venepuncture and Central Line Insertion

The mean APACHE II score of the patients undergoing venepuncture was 16.8 (range 11 - 26). There was no significant difference between the plasma cortisol concentrations before and after insertion of a cannula into an antecubital fossa vein in the 10 cases investigated. Mean [SD] plasma cortisol concentration from central line sampling was 873 [286] nmol/L compared to 889 [301] nmol/L after insertion of the cannula.

The mean APACHE II score of the 12 patients undergoing central line insertion was 17.4 (range 15-24). In 2 cases a single cortisol measurement was unavailable. Central line insertion was associated with an increase in plasma cortisol concentration in 11 of the 12 patients investigated [Fig A1, Table A1].

Table A1. Mean [SD] plasma cortisol concentration following central line insertion.

TIME [mins]	PLASMA CORTISOL CONCENTRATION [nmol/L].		
	0	30	60
SEDATED [N=7]	998 [211]	1071 [212]	1042 [201]
UNSEDATED [N=5]	823 [296]	906 [279]	1051 [284]
TOTAL [N=12]	911 [271]	989 [261]	1047 [246]

The overall increase in plasma cortisol concentration between the basal and 60 minute samples in sedated and unsedated patients combined was significant [mean difference (MD) = 133 nmol/L, 95% CI = 19 - 247]. The increase in plasma cortisol concentration between the basal and 60 minute samples reached significance in unsedated patients [MD = 228 nmol/L, 95% CI = 80 - 400]. In sedated patients the maximum mean plasma cortisol concentration was seen at 30 minutes, but the increase from basal concentration was not significant [MD = 68 nmol/L, 90% CI = -12 - 145].

Intravenous Fluids and Blood Transfusion

The mean APACHE II score of the 24 patients studied was 21.2 (range 9 - 34). All cortisol measurements were available. Plasma cortisol concentration was not significantly altered during the intravenous saline infusion [Fig A2, Table A2] . In 5 of the 6 cases investigated, plasma cortisol concentration fell following the saline infusion. The fall in cortisol concentration between 2 and 4 hours reached statistical significance [MD = 210 nmol/L, 99% CI = 7 - 413]. The decrease in cortisol concentration was significantly greater than in the corresponding period in control patients [MD = 160 nmol/L, 95% CI = 35 - 286]. There was no significant change in plasma cortisol concentration during or after intravenous albumin infusion [Fig A2, Table A2].

Table A2. Mean [SD] plasma cortisol concentration following intravenous infusions of 0.9% saline and 20% albumin.

TIME [hours]	PLASMA CORTISOL CONCENTRATION [nmol/L]			
	0	1	2	4
SALINE:				
Mean [SD]	1087 [551]	1059 [549]	1128 [643]	918 [541]
ALBUMIN:				
Mean [SD]	991 [381]	1023 [400]	1022 [362]	1119 [430]

There was an increase in plasma cortisol concentration during the first hour of the intravenous infusion of TPN in all patients studied [Fig A3, Table A3]. This increase reached statistical significance [MD = 121 nmol/L, 95% CI = 28 - 214]. The cortisol increase from basal to 1 hour was significantly greater than the corresponding increase in control patients [MD = 97 nmol/L, 95% CI = 2 - 193]. Plasma cortisol levels fell significantly between 1 and 4 hours after commencing the infusion [MD = 126 nmol/L, 95% CI = 33 - 298]. There was no significant change in plasma cortisol concentrations during or after blood transfusion [Fig A3, Table A3] or in controls [Table A3].

Table A3. Mean [SD] plasma cortisol concentration following infusions of total parenteral nutrition or blood or in controls.

TIME [hours]	PLASMA CORTISOL CONCENTRATION [nmol/L]			
	0	1	2	4
TPN:				
Mean [SD]	830 [373]	951 [394]	827 [296]	767 [301]
BLOOD:				
Mean [SD]	1046 [444]	1008 [388]	1047 [454]	1003 [638]
CONTROLS:				
Mean [SD]	975 [366]	999 [355]	986 [388]	938 [349]

Enteral Fluids

The mean APACHE II score of patients investigated was 14.2 (range 9 - 21). All cortisol measurements were available. Plasma cortisol concentrations increased during infusions of full strength enteral feed in 5 of the 6 patients investigated [Figs A4, Table A4]. The increase reached significance between the basal measurements and both the 1 hour [MD = 92 nmol/L, 95% CI = 3 - 180] and the 2 hour [MD = 154 nmol/L, 99% CI = 69 - 238] measurements. The fall in cortisol concentration between 2 and 4 hours did not reach significance [MD = 110 nmol/L, 95% CI = -24 - 244]. Plasma cortisol concentrations fell during and after the infusion of enteral water in 4 of the 6 patients investigated [Fig A4, Table A4].

Table A4. Mean [SD] plasma cortisol concentration following infusion of full strength enteral feed or enteral water.

TIME [hours]	PLASMA CORTISOL CONCENTRATION [nmol/L]			
	0	1	2	4
FEED:				
MEAN [SD]	766 [281]	858 [225]	919 [302]	796 [250]
WATER:				
MEAN [SD]	752 [188]	725 [239]	676 [182]	642 [138]

Physiotherapy

The mean APACHE II score of patients studied was 17.4 (range 12 - 29). A single cortisol measurement was missing in 2 cases. There was a non-significant increase in plasma cortisol concentration in response to physiotherapy in 6 of the 8 patients studied [Fig A5, Table A5].

Table A5. Mean [SD] plasma cortisol concentration following physiotherapy.

TIME [minutes]	PLASMA CORTISOL CONCENTRATION [nmol/l]		
	0	30	60
MEAN [SD]	1031 [510]	1099 [467]	1012 [494]

Haemodialysis

The mean APACHE II score of patients studied was 21.6 (range 16 - 34). All cortisol measurements were available. Mean duration of haemodialysis was 126 minutes (range 46 - 243 mins). Plasma cortisol concentration increased after 30 minutes dialysis in 6 patients and after 1 hour in all patients [Fig A6]. The difference in mean plasma cortisol concentrations was significant at 1, 2 and 4 hours after commencement of dialysis [MD at 1 hour = 204 nmol/L (99% CI = 35 - 374), MD at 2 hours = 235 nmol/L (95% CI = 37 - 433), MD at 4 hours = 148 nmol/L (95% CI = 30 - 265), Table A6]. There was no change in the concentration of cortisol in the control group during the study period [Fig A7, Table A6].

Table A6. Mean [SD] plasma cortisol concentration before, during and after haemodialysis.

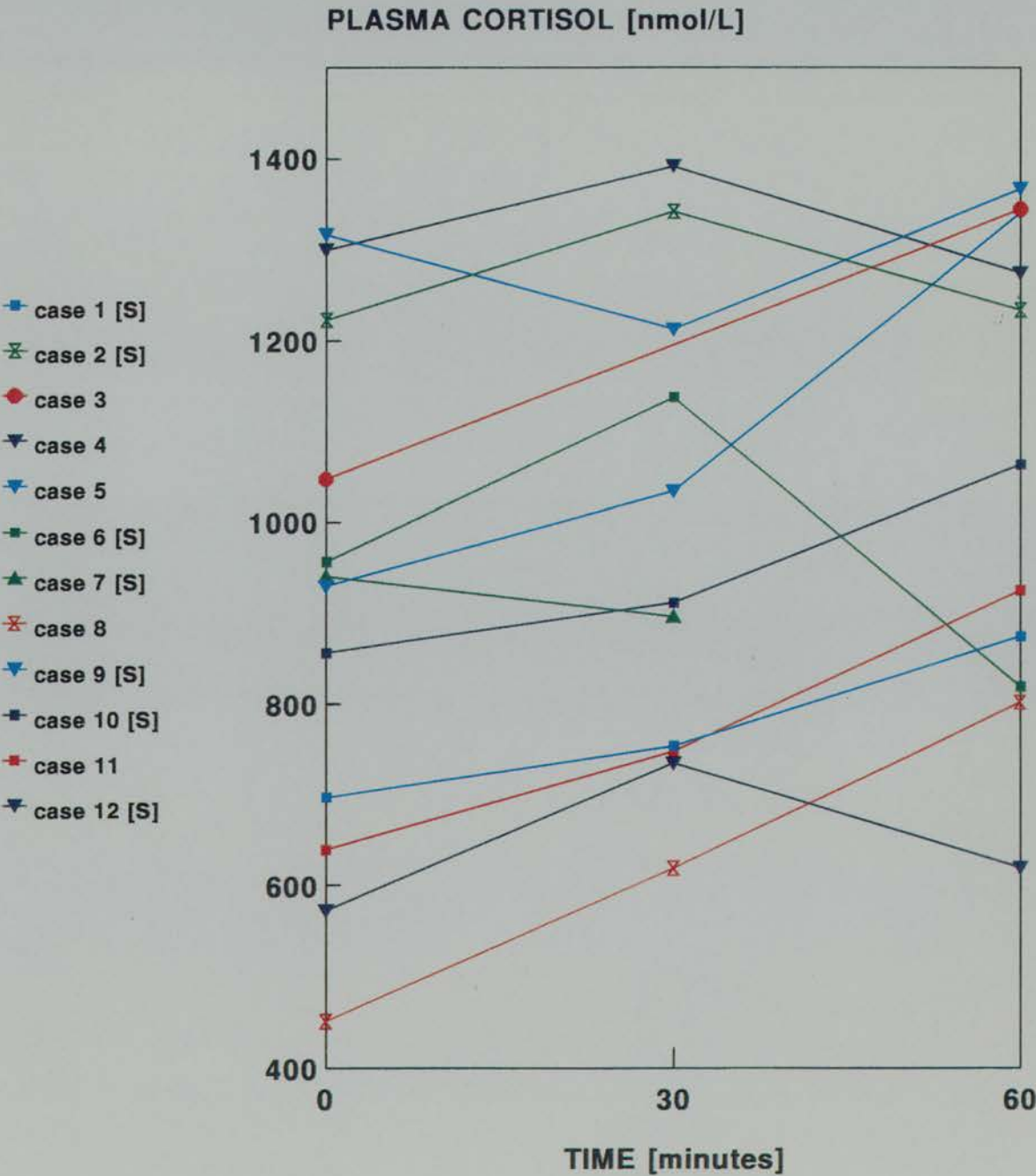
TIME [mins]	MEAN/SD PLASMA CORTISOL CONCENTRATION [nmol/L]				
	0	30	60	120	240
CASES	983 [235]	1064 [303]	1188 [318]	1218 [367]	1131 [276]
CONTROLS	1056 [216]	1039 [301]	1048 [298]	1015 [316]	983 [351]

Mean arterial blood pressure was lower than the basal pressure after 1 hour of dialysis in all patients [Fig A8]. The mean level was significantly lower than baseline at 30, 60, 90, 120, 150 and 180 minutes after commencement of dialysis [$P < 0.01$ at 60 and 90 mins, otherwise $P < 0.05$, Table A7]. Control measurements of MAP showed no significant change over the 4 hour period [Fig A9, Table A7]. Absolute MAP was lower in dialysis patients than controls from 30 to 180 minutes after commencement of dialysis. There was a significant correlation between the extent of the fall in MAP and the rise in plasma cortisol concentration 60 minutes after commencement of dialysis [$r = 0.774$, 95% CI 0.153 - 0.957].

Table A7. Mean [SD] mean arterial pressure during and after haemodialysis and during control period.

TIME [mins]	MEAN ARTERIAL PRESSURE [mm Hg]								
	0	30	60	90	120	150	180	210	240
CASES	99[14]	93[15]	90[13]	92[15]	92[18]	92[16]	94[17]	98[16]	99[15]
CONTROLS	98[13]	97[13]	97[11]	98[11]	96[13]	96[11]	97[10]	97[11]	97[13]

FIG 1. PLASMA CORTISOL RESPONSE TO CENTRAL LINE INSERTION



MISSING DATA IN CASES 3 AND 7.

FIG 2. PLASMA CORTISOL RESPONSE TO INFUSIONS OF ALBUMIN AND SALINE.

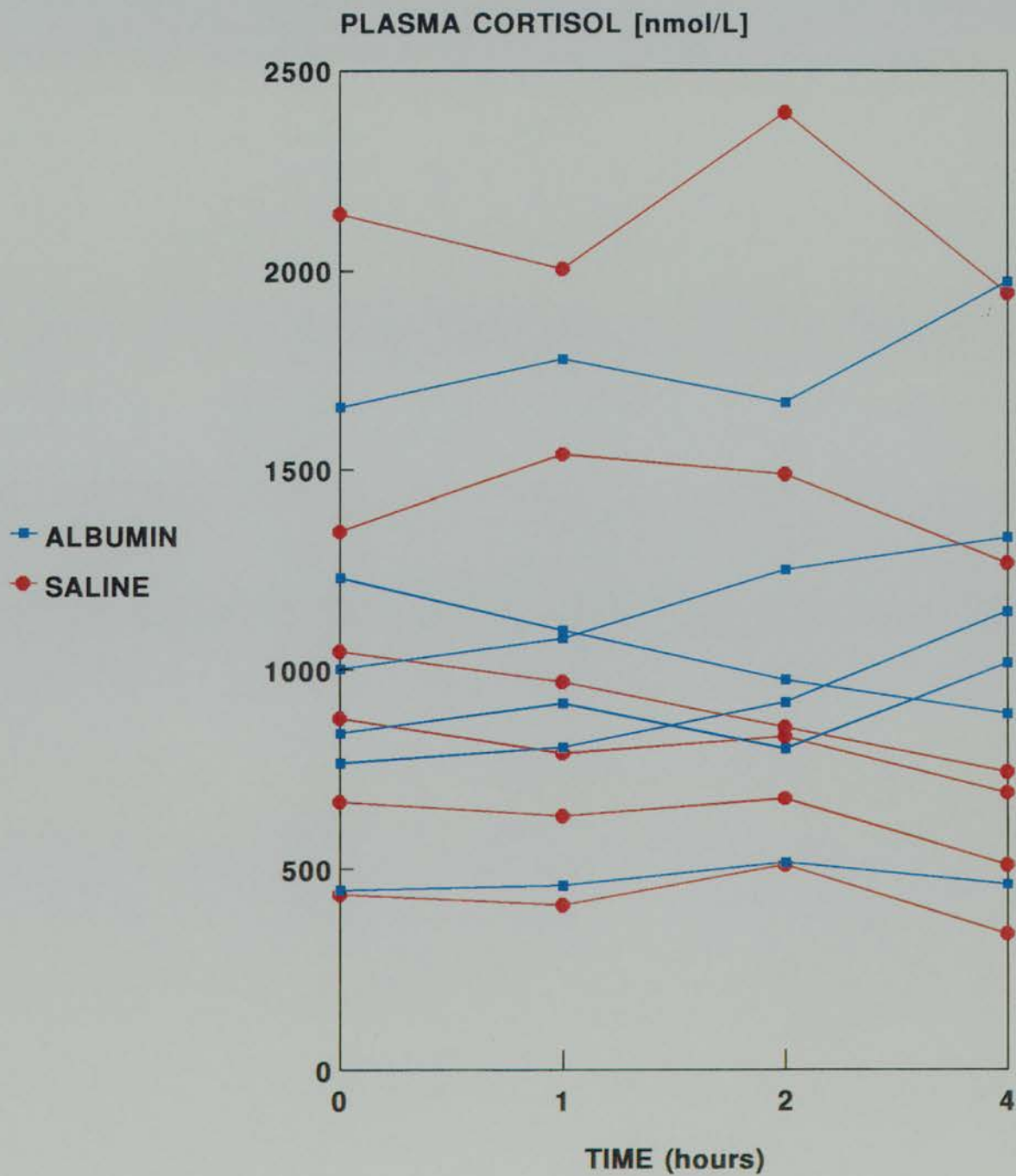


FIG 3. PLASMA CORTISOL RESPONSE TO INFUSIONS OF BLOOD AND TPN

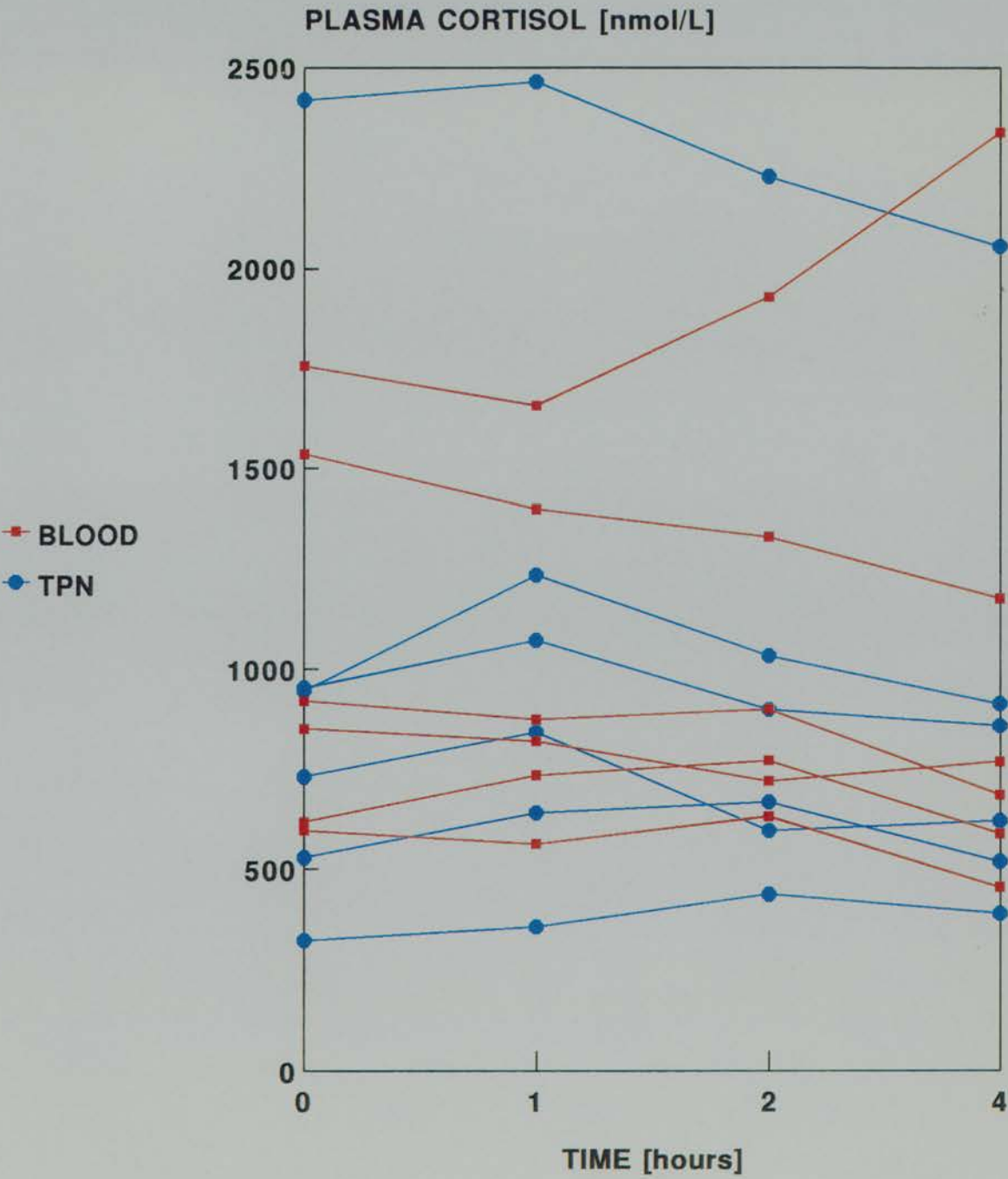


FIG 4. PLASMA CORTISOL RESPONSE TO INFUSIONS OF FULL STRENGTH ENTERAL FEED AND WATER

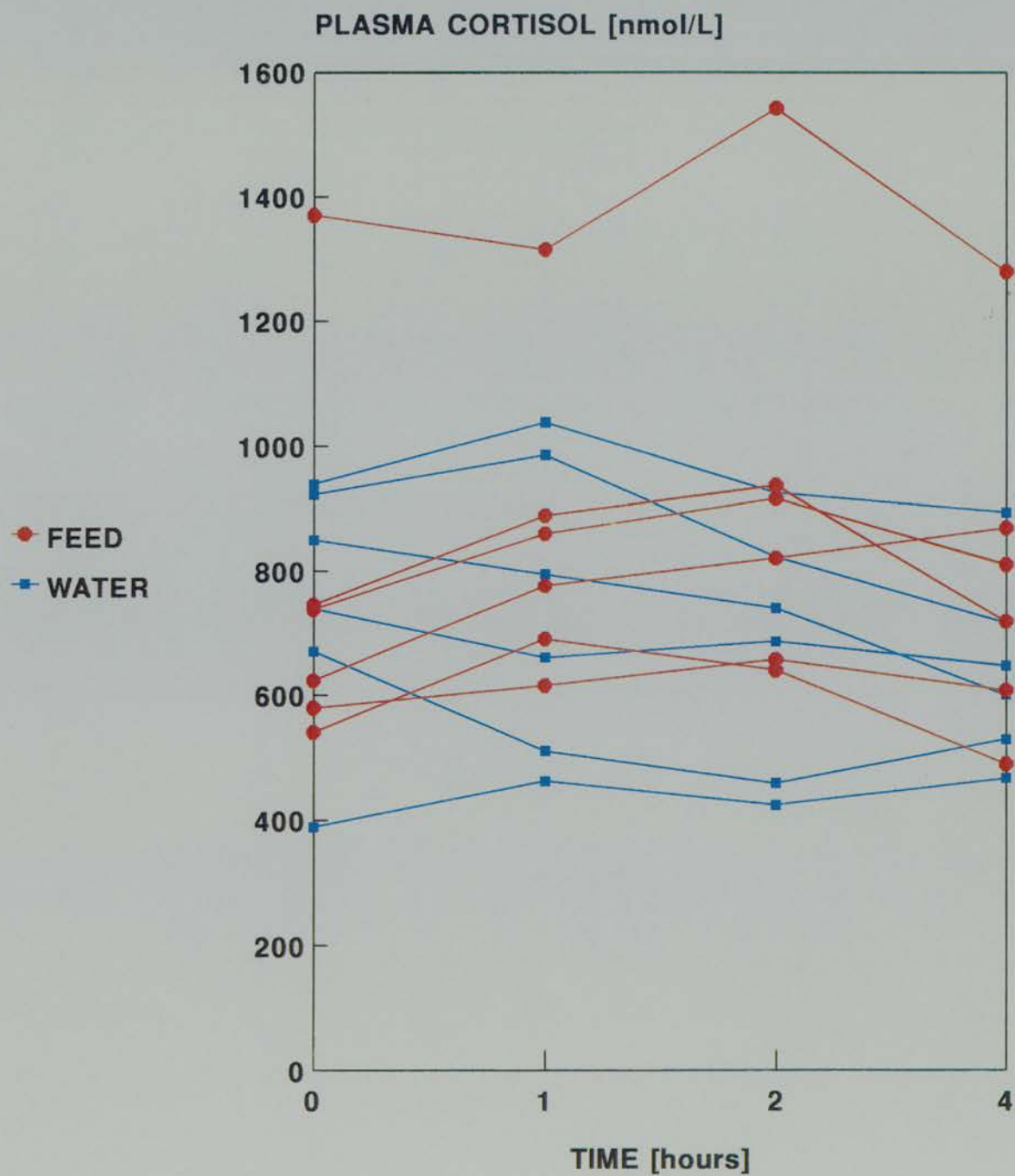
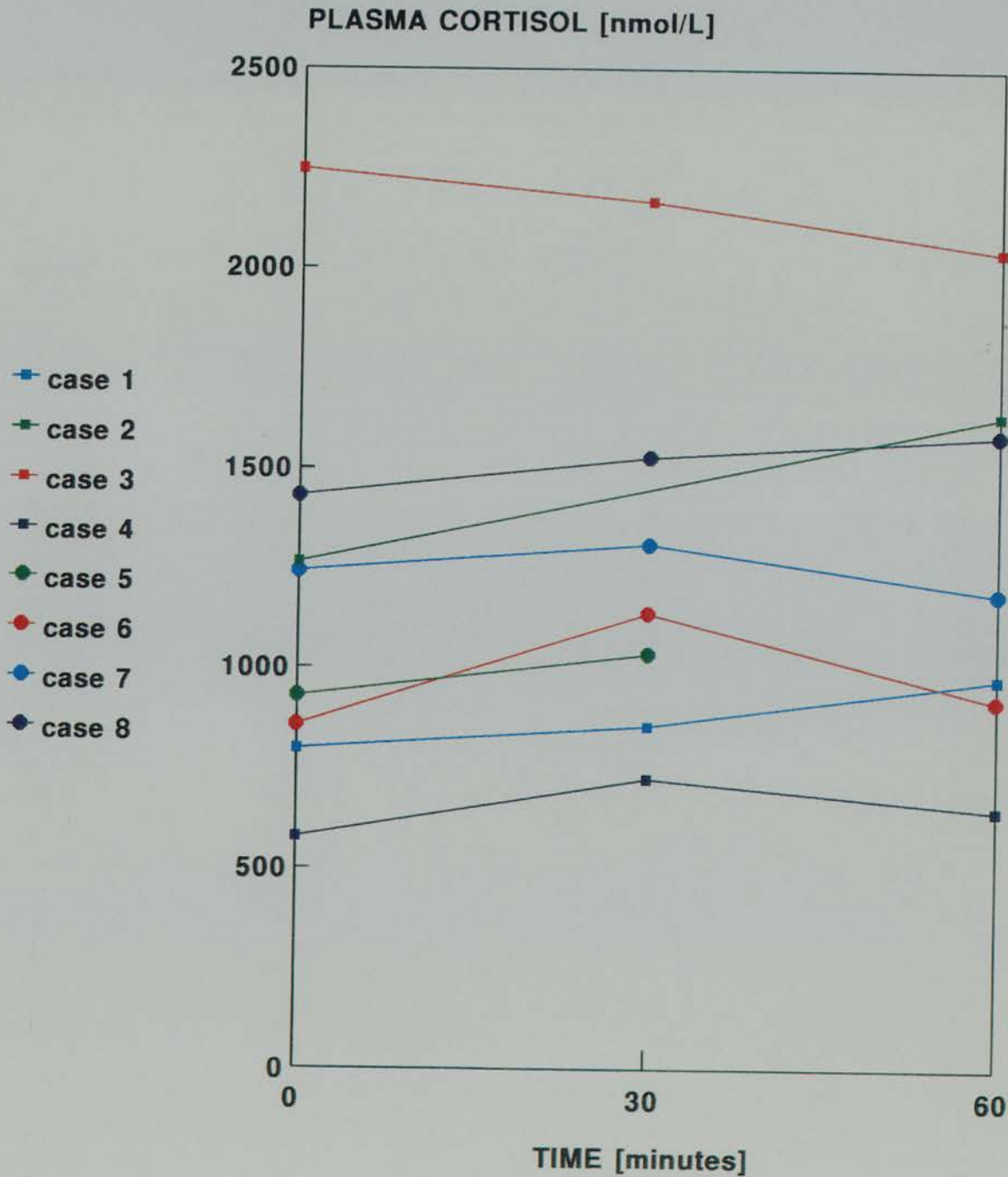


FIG 5. PLASMA CORTISOL RESPONSE TO PHYSIOTHERAPY



MISSING DATA IN CASES 2 AND 5.

FIG 6. PLASMA CORTISOL CONCENTRATION DURING AND AFTER HAEMODIALYSIS

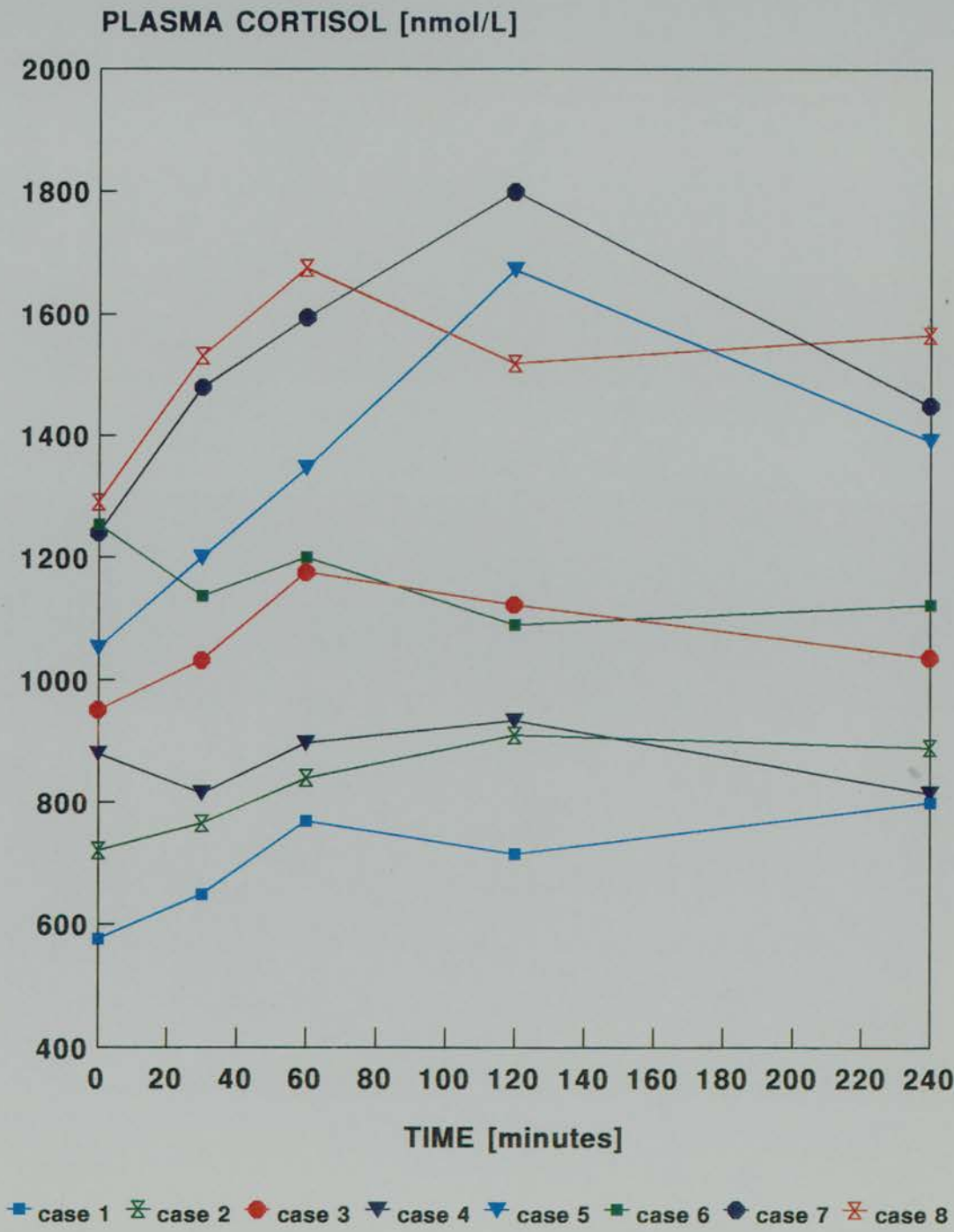


FIG 7. PLASMA CORTISOL CONCENTRATION DURING HAEMODIALYSIS CONTROL PERIOD

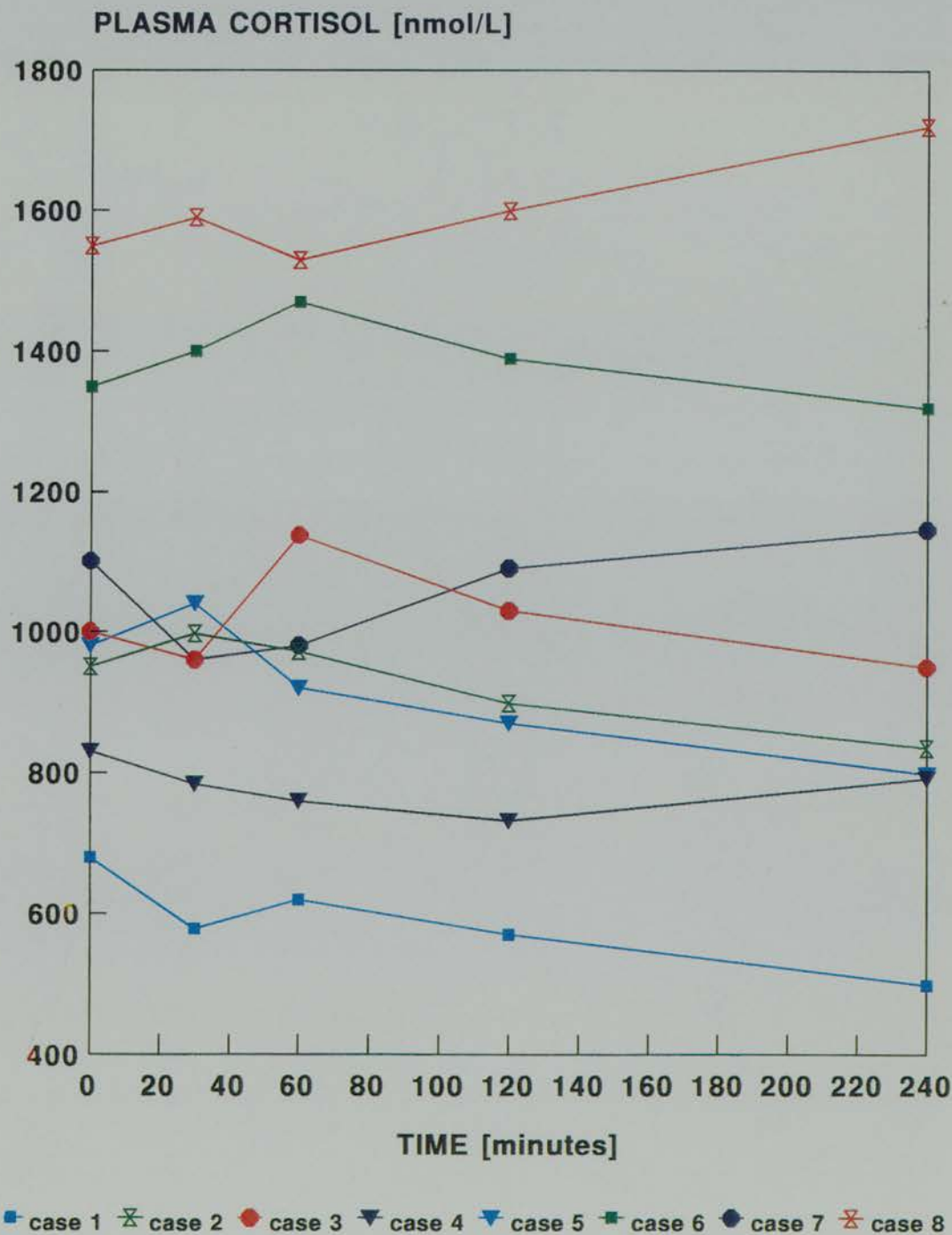


FIG 8. MEAN ARTERIAL PRESSURE DURING AND AFTER HAEMODIALYSIS

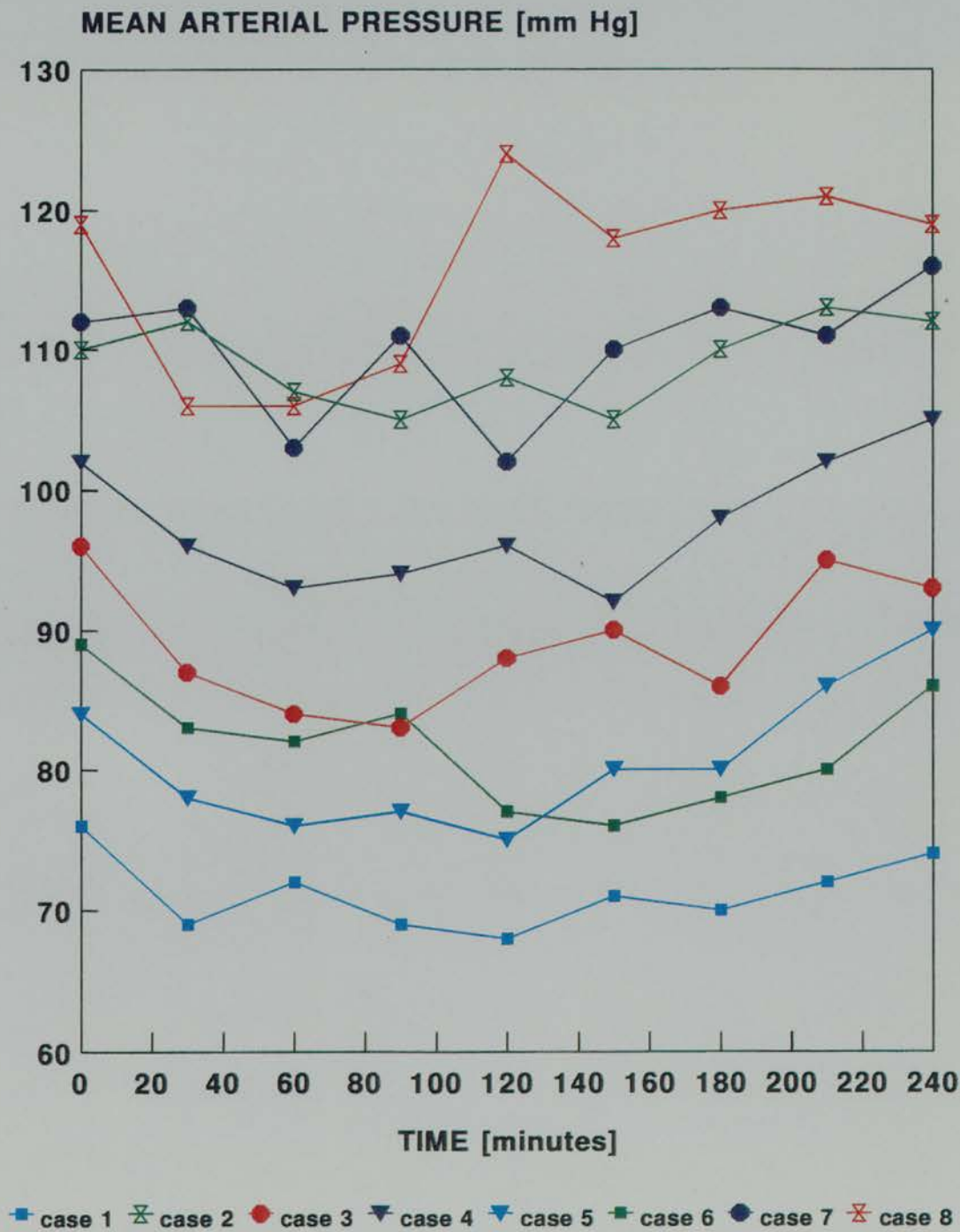
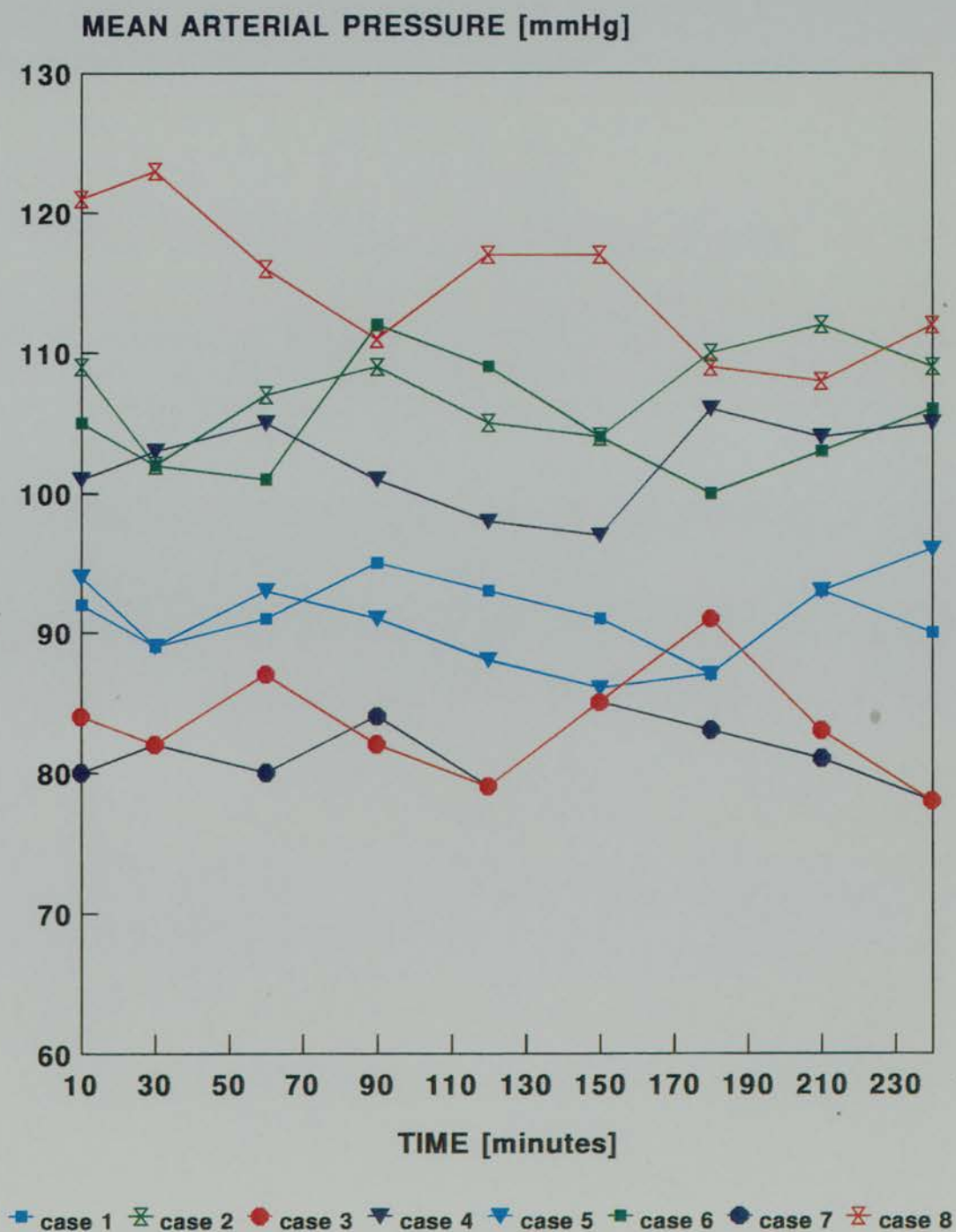


FIG 9. MEAN ARTERIAL PRESSURE DURING
HAEMODIALYSIS CONTROL PERIOD



(5) Discussion

Venepuncture and Central Line Insertion

It is recommended that all forms of stress be avoided when measuring plasma cortisol concentration. Venepuncture is a "stressful" experience for many patients, and has been shown to increase cortisol concentrations for 1 to 2 hours (Van Cauter and Honinckx, 1985). No significant effect was noted in this study, although only a small number of patients were investigated, and blood was sampled within 1 or 2 minutes of venepuncture. The intention was to investigate whether cortisol measurements made following venepuncture would differ from those made at the same time in the same patient via central venous access. No difference was demonstrated. Repeated performance of stressful tasks reduces the associated cortisol response [Domanski, 1957], and it is possible that, since the study patients had undergone numerous venepunctures in the days immediately prior to being studied, the procedure was no longer stressful. However, it is more likely that by sampling so soon after venepuncture, a cortisol stress response was missed. This is not a problem for single measurements, but might be a source of bias in dynamic tests of adrenocortical function involving repeated blood sampling over a period of time.

Central venous line insertion is a longer, more formal, and more painful procedure than venepuncture, despite the use of local anaesthetic. The line is secured using sutures, and the procedure takes at least 15 minutes. A consistent and significant rise in plasma cortisol concentration was seen following central line insertion. The response was greater in unsedated than sedated patients. Sedation with benzodiazepines reduces, but does not abolish, the cortisol response to emotional and surgical stress by limiting the ACTH response (Nilsson, 1990). The cortisol response to surgical procedures requiring general anaesthesia is markedly reduced by also giving epidural anaesthesia (Nielsen et al, 1989), suggesting that neural mechanisms are involved in ACTH release during surgery. Sedation will clearly reduce the conscious stress response, but will not abolish the neurally mediated nociceptive response.

Intravenous Fluids and Blood Transfusion

The majority of intensive care patients require intravenous hydration, and rapid fluid replacement may sometimes be necessary. It is conceivable that rapid administration of crystalloids might reduce plasma corti-

sol concentration for a short period by a process of dilution. Such an effect might be more pronounced with concentrated albumin solution which draws fluid into the vascular space. Albumin is also involved in the protein binding of cortisol.

Saline administration did not significantly alter plasma cortisol concentration during the infusion. A non-significant rise in cortisol concentration occurred, suggesting that dilution is not a problem at the infusion rate studied. There was a significant fall in cortisol concentration over the 2 hours following the infusion, which also occurred to a non-significant extent in control cases. The reasons for this are unclear, and any detailed analysis would require more cases. However, despite the small number of patients studied, it can be concluded that at a constant infusion rate of 250 ml/hour intravenous saline does not significantly alter plasma cortisol concentration during the infusion.

Infusion of neither albumin nor blood altered plasma cortisol concentration. It is possible that by investigating only 6 patients a small change might have been missed. The effect of blood transfusion might vary depending upon the individual. In the event of a minor blood transfusion reaction, it is likely that the cortisol concentration would rise.

Nutrition

Infusion of TPN was associated with a significant rise in plasma cortisol concentration after 1 hour, followed by a fall. The initial rise occurred in all patients. Eating increases cortisol secretion (Follenius et al, 1982), and in healthy patients, peaks in plasma cortisol concentration, superimposed on the diurnal rhythm, occur after each meal. The reasons for this response to food are unclear. There is no published data on the cortisol response to intravenous nutrition. This small investigation suggests that the response is similar to that following oral food intake. TPN infusions are not continuous, and are stopped for at least 4 hours each day. It is possible, therefore, that they might interfere with dynamic tests of adrenocortical function.

A significant proportion of ICU patients receive enteral nutrition. This is frequently intermittent, and may alter cortisol secretion. In patients given enteral water there was no significant change in plasma cortisol secretion. In patients receiving enteral nutrition there was a significant increase in plasma cortisol concentration during the infu-

sion, followed by a fall to pre-infusion levels 2 hours after the infusion had finished. Whether or not the increase in cortisol concentration is maintained during longer infusions of enteral nutrition was not investigated.

Physiotherapy

Physiotherapy is carried out at least once daily on all ventilated and the majority of non-ventilated ICU patients. The procedure requires oropharyngeal and tracheobroncheal suction. Despite the unpleasant nature of the procedure, no significant rise in cortisol concentration was detected in the patients investigated. The basal cortisol concentrations were high, and given there significant intra-assay variation at such levels, a cortisol response to physiotherapy of up to 200 nmol/l could easily have been missed.

Haemodialysis

Acute renal failure occurs in 30% of patients in the ICU. These patients require regular haemodialysis which may interfere with adrenocortical function. Previous research on this topic has been performed in otherwise healthy patients suffering from chronic renal failure. Dialysis must remove significant amounts of free cortisol, but does not remove cortisol binding globulin or albumin (Thysell et al, 1979). Concentrations of ACTH and cortisol increase during dialysis, falling to normal after 24 hours (Maher et al, 1965; Grekas et al, 1983). Adrenal responsiveness to ACTH has been reported to be normal (Grekas et al, 1983; Siamopoulos et al, 1988) and impaired (Mrinak et al, 1981) in chronic renal failure. There are no published data on patients suffering from acute renal failure.

Plasma cortisol concentration increased in all patients studied during dialysis, and levels remained high at 4 hours after commencement of dialysis. The reasons for this response are unclear. Since plasma cortisol had increased within 30 minutes of commencing dialysis, it seems unlikely that a reduction in urea concentration was responsible. Acute reduction in blood pressure is associated with increased cortisol concentration (Grassler et al, 1990). In the patients studied haemodialysis coincided with a significant fall in MAP. Blood pressure returned to pre-dialysis levels after completion of dialysis. The hypotensive response to haemodialysis is well recognised in ICU patients, and often

limits the rate of blood flow into the dialysis machine, and hence, the length of dialysis. The extent of the fall in MAP correlated with the increase in cortisol concentration. This dose response effect suggests that the hypotension may cause the cortisol response.

(6) A Protocol for the Investigation of Adrenocortical Function During Critical Illness

The results discussed above are based on investigation of small numbers of patients in whom cortisol concentrations were raised to a level where there is considerable intra-assay variability. For the interventions which did not appear to alter plasma cortisol concentrations, a minor cortisol response cannot be excluded. However, it is likely that those interventions which led to the greatest alteration in cortisol concentration have been correctly identified. It is therefore possible to define conditions for the investigation of adrenocortical function in which some of the major confounding influences can be excluded:

- 1) Minor surgical procedures and the insertion of invasive monitoring equipment, such as central and arterial lines, should be avoided during tests of adrenocortical function and for 2 hours prior to the test.
- 2) Physiotherapy, including tracheobronchial suction, may be performed before and during investigations.
- 3) The following intravenous fluid regimen will be permitted during investigation: a) Saline infusions at rates equal to, or less than, 250ml per hour; b) Infusions of 20% albumin at rates equal to, or less than, 50 ml per hour; c) Transfusion of cross-matched whole blood at rates of equal to, or less than, 200ml per hour; d) Infusions of total parenteral nutrition and enteral nutrition should be stopped 2 hours prior to investigation and for the duration of the tests.
- 4) No investigations should be carried out during or in the 2 hours following haemodialysis, although the timing of investigation need not be influenced by the blood urea concentration.

It could be argued that most of the above recommendations are common sense. However, the finding that intravenous fluids, blood and physiotherapy may be given during dynamic tests of adrenocortical function without completely invalidating the results is important. A protocol which excluded all such interventions would be very difficult to adhere to in practice in studies of critically ill patients.